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UPDATED BIBLIOGRAPHIC REVIEW OF THE POSSIBLE FUNCTIONAL EFFECTS OF DIFFERENT LEMON NUTRIENTS

(*C. limon*)

FINAL REPORT

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UPDATED BIBLIOGRAPHIC REVIEW OF THE POSSIBLE FUNCTIONAL EFFECTS OF DIFFERENT LEMON NUTRIENTS (C. limon)

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Executive summary

Throughout history, lemon has been considered as a fruit with a large amount of functional properties that are beneficial to the body due to its rich chemical composition.

However, many of these functional effects have been assumed to be true as a result of tradition, rather than because of specific studies performed on the matter.

The aim of this report is to conduct a bibliographic review on the scientific evidence regarding the functionality and biological effectiveness, of both lemon and its essential oil.

The most recent pharmacological studies show the healthy biological activities of the *C. limon* fruit, as well as its increasing application in cosmetology and food production.

Component of *C.limon*	Biological Activity	Applications in the Food Industry	Applications in the Cosmetic Industry
Extract or juice	Anticancer Antioxidant Anti-inflammatory Antibacterial Antifungal Antiviral Antiallergic Hepatoregenerative Diabetes prevention Dietary activity Effects on the cardiovascular system Effects on the nervous system Effects on the skeletal system	Gelling Thickener Texturizer Emulsifier Stabilizer in dairy products Seasoning Flavouring Preservative Acidulant	Anti-acne Antioxidant Increase collagen production Anti-wrinkle

Functionality and biological effectiveness of *C. lemon* extract or juice

Component of *C.limon*	Biological Activity	Applications in the Food Industry	Applications in the Cosmetic Industry
Essential oil	Anticancer Anti-inflammatory Antibacterial Anticaries Antifungal Antiviral Antiparasitic Hepatoprotective and detoxifying Diabetes prevention Anti-obesity Effects on the digestive system Cholesterol reduction Effects on the nervous system	Flavouring Preservative	Depigmenting effect Promoter of active substances penetration into the skin Natural preservative Antibacterial activity Fungistatic effect Diabetic wound regeneration

Functionality and biological effectiveness of *C. lemon* essential oil

In addition, general aspects about its botanical characteristics, chemical composition or metabolic profile are described throughout the document. Traditional applications of lemon documented in scientific literature are cited as well.

However, despite the wide variety of nutrients present in lemon, studies have mainly focused in the most abundant ones such as Vitamin C or D-limonene, while innumerable compounds that are potential nutrients could show functionality and biological activity at greater concentrations than the ones found in lemon juice, such as hesperidin among others.

On the other hand, many of the compounds found in lemon juice could present difficulties in their absorption in the intestine and demonstrate the expected physiological effect due to the matrix and the environment in which they are found. Accordingly, future studies should focus their efforts on modifying of the oral administration systems and conducting clinical trials that prove the safety and efficacy in groups of human individuals, improving the payload of nutrients, their *in vivo* stability, their biodistribution and the efficacy of biological functionality.

1 Title

Updated bibliographic review of the possible functional effects of different lemon nutrients (*C. limon*)

2 Introduction

Citrus limon (L.) Burm. f. is a tree with evergreen leaves and yellow edible fruits of the Rutaceae family.

The main primary material of *C. limon* is its fruit, particularly the essential oil and the juice obtained from it. The *C. limon* fruit stands out due to its well-known nutritional properties, but it is worth mentioning that its valuable biological activities are underestimated in modern physiotherapy and cosmetology[1].

Prior to the discovery of Vitamin C, *C. limon*'s fruit juice (lemon juice) was classically used as a remedy against scurvy[2]. This common use of *C. limon*, known since ancient times, has nowadays been sustained by numerous scientific studies. Other traditional uses of lemon juice include treatment against high blood pressure, common cold and irregular menstruation. On the other hand, *C. lemon* essential oil is a folk remedy against cough [3–5]. In addition to being rich in Vitamin C, which helps prevent infections, the juice is traditionally used to treat scurvy, sore throat, fevers, rheumatism, high blood pressure and chest pain[6].

In Trinidad, a mixture of lemon juice with alcohol or coconut oil has traditionally been used to treat fever, common cold cough and high blood pressure. Moreover, juice or grated skin mixed with molasses has been used to remove excess body water, and juice mixed with olive oil has been administered to battle uterine infection and kidney stones[4]. According to traditional Indian medicine, *C. limon* juice can induce menstruation; the traditionally recommended dose for this purpose is two teaspoons taken twice a day[5].

Currently, valuable scientific publications focus on the increasingly recognized pharmacological actions of *C. limon* extract, juice and essential oil. Some of them demonstrate antibacterial, antifungal, anticancer, anti-inflammatory hepatoregenerative and cardioprotective activities, to name a few[7–11].

C. limon's pharmacological potential is determined by its rich chemical composition. The most important secondary metabolite group in fruit includes flavonoids and other compounds such as phenolic acids, carboxylic acids, amino acids and vitamins. The main compounds of essential oil are monoterpenoids, especially C-limonene. These valuable chemical compounds are the reason for the important position of *C. limon* in the food and cosmetic industries[12–14].

It is worth emphasizing that *C. limon*'s traditional uses may be rooted in the culture and customs of certain regions or countries, but its effectiveness must be demonstrated by scientific evidence.

The objective of this systematic review is to provide an overview of scientific papers and an in-depth analysis of the latest research related to *C. limon* as a valuable and important plant species in pharmacy, cosmetology and the food industry. Furthermore, the most relevant biotechnological research is presented.

3 The genus *Citrus*

The genus *Citrus* is one of the most important taxonomical subunits of the *Rutaceae* family. The fruits produced by the species belonging to this genus are colloquially called 'citrus'. Citrus are commonly known for their valuable nutritional, pharmaceutical and cosmetics properties. The *Citrus* genus includes perennials, shrubs or trees (3-15 metres tall). Its leaves are leathery, ovoid or elliptical. Some of them have spikes. Flowers grow individually in the axils of the leaves. Each flower has five petals, white or reddish. The fruit is a *hesperidium* berry. The species of the *Citrus* genus are naturally found in warm and temperate climates, mainly in the Mediterranean region. They are usually sensitive to frost[2].

One of the best known and most exploited species of the *Citrus* genus is the lemon: *Citrus limon* (L.) Burm. f. (Latin synonyms: *C.* × *limonia, C. limonum*). Other important species included in this taxonomic unit are: *Citrus aurantium* ssp. *Aurantium* (bitter orange), *Citrus sinensis* (Chinese orange), *Citrus reticulata* (mandarin), *Citrus paradise* (grapefruit), *Citrus bergamia* (orange bergamot), *Citrus medica* (citron), among others. A team of scientists for the University of California (Oakland, California, EEUU) analysed the origin of several species of the *Citrus* genus, *C. limon* among them. They found that *C. limon* had formed as a result of the combination of two species: *C. aurantium* and *C. medica*[15]. The metabolite profiles of C. *limon, C. aurantium* and C. *medica* were evaluated by scientists from the Southwest University of China (Chongqing, China)[16]. They demonstrated that *C. limon* has a shorter phylogenetic distance with *C. aurantium* and *C. medica* in comparison to other *Citrus* species. These studies showed that *C. limon* was probably a hybrid of *C. medica* and *C. aurantium,* as initially suspected[16].

The botanical classification of the *Citrus* genus species is very complex due to the frequent formation of hybrids and the introduction of numerous crops through cross pollination. Hybrids are produced to obtain fruits with valuable organoleptic and industrial properties, including seedless fruit, high juiciness and new flavours. Advanced molecular techniques are often necessary to identify older varieties, hybrids and crops. *C. limon*, like many other prolific citrus species, results in numerous varieties, crops and hybrids, presented in Tables 1 and 2[17].

Name	Origin	Growing region	Characteristics
C. limon 'Primofiori' (*C. limon* 'Fino', *C. limon* 'Mesero', *C. limon* 'Blanco')	Spain	Spain	A productive variety with spines. Fruits have a spherical or oval shape, with a small wart at the end.
C. limon 'Berna'—(*C. limon* 'Verna', Verma lemon)	Spain	Spain	The specimens are large, without spines. It blooms two to three times a year. Fruits from individual harvests differ in properties and are called 'Cosecha' (main collections), 'Secundus' and 'Rodrejos'.
C. limon 'Bearss' (*C. limon* 'Sicilian', lemon Bearss)	Florida	Florida, Brazil	It grows quickly and is very productive. It has aromatic flowers, juicy fruit and a high sensitivity to low temperatures.
C. limon 'Femminello'	Italy	Italy	A very productive variety. It blooms and bears fruit throughout the year.
C. limon 'Genova' (*C. limon* 'Genoa')	Italy	California, Florida, Chile	Spike-free trees, resistant to cold with dense foliage. Yellow fruits with a marked tip have a smooth and thin pericarp.
C. limon 'Interdonato'	Italy	Italy	It has large, oblong, cylindrical pointed fruit. Pericarp strongly adheres to the fruit; it is thin, smooth, and yellow. With few seeds.
C. limon 'Lisbon'	Portugal	California, Arizona, Australia, Uruguay	It has long spines, thick skin, pink flowers, and pale-yellow flesh.
C. limon 'Monachello'	Italy	Italy	The main advantage of this variety is high resistance to the disease caused by *Phoma tracheiphila*.
C. limon 'Santa Teresa' (*C. limon* 'Feminello Santa Teresa', *C. limon* 'Italian')	Italy	Italy, North-West Argentina, Turkey	Pericarp, contains a large amount of essential oil. The fruit contains a large amount of juice. This variety is resistant to storage and transport.
C. limon var. Variegata (*C. limon* 'Eureka' var. Variegated, Pink-fleshed lemon.	California, Sicily	California	Established as a result of the intrinsic mutation of C. limon 'Eureka' in 1931. It has pulp and juice of a pink shade. The fruits are yellow with green stripes and variegated leaves.
C. limon 'Villafranca'	Sicily	Florida, Israel, Northwest Argentina	It has pulp and juice of a pink shade. The fruit is yellow with green stripes.

Table 1. *C. limon* cultivars.

One of the oldest still preserved botanical sources describing species of the genus *Citrus* is the "Monograph on Wên-chou oranges" by Han Yanzhi of 1178[18,19]. Other historical works describing citrus species are "Nürnbergische Hesperides" from 1708 and "Traité du Citrus" from 1811. Historically, one of the best-known classifications of citrus species is "Histoire Naturelle des Orangers" from 1818. American botanist Walter Tennyson Swingle (1871-1952) had a particularly significant impact on the current taxonomy of the *Citrus* genus. He is the author of up to 95 botanical names for species of this genus. Currently, the systematics of the species of the genus *Citrus* are based on studies of molecular markers and other DNA analysis technologies that continue to provide new information to this day[20].

Name	Origin	Characteristics
C. limon 'Lemonime'	Hybrid *C. limon* and *C. aurantifolia*	It has fruit larger than limes (*C. aurantifolia*).
C. limon 'Lumia'	Hybrid *C. maxima* and *C. medica*, subsequently hybridized with *C. limon*	The fruit resembles a pear. It can reach large sizes.
C. limon 'Ponderosa'	Hybrid *C. limon* and *C. medica*	Fruits with a pear-shaped and thick pericarp.
C. limon 'Volkamer'	Hybrid *C. limon* and *C. aurantium*	Specimens smaller than *C. limon*. The fruit has few seeds and a thick, rough, light reddish pericarp. The flesh and juice are yellow-red. The hybrid is resistant to many diseases.

Table 2. Hybrids of *C. limon*.

3.1 Botanical characteristics and presence of *C. limon*

Citrus limon (L.) Burm. f. (lemon) is a tree that reaches 2,5 - 3 metres in height. It has evergreen lanceolate leaves. The bisexual flowers are white with a purple tint at the edges of the petals. They gather in small clusters or are produced individually, growing in leaf axils. The fruit is an elongated, oval, and pointed green berry that turns yellow during ripening. Inside, the berry is filled with a juicy pulp divided into segments. The pericarp of *C. limon* is made of a thin, wax-covered exocarp, under which lies the outer part of the mesocarp, also known as the flavedo. This part contains oil vesicles and carotenoid pigments. The inner part of the mesocarp, also known as the albedo, is made of a spongy white parenchyma tissue. The endocarp, or 'fruit flesh', is divided into segments by the spongy, white tissue of the mesocarp[2].

The *C. limon* tree preferentially inhabits sunny places. It grows in loamy, well-drained and moist soils with a wide range of pH[1,2]. The location of the original natural habitat of *C. limon* is not exactly known[1,21]. However, it is considered to be native to north-western or north-eastern India[2,17].

C. limon is primarily recognized as a cultivated species. It has been cultivated in southern Italy since the 3rd century AD, and in Iraq and Egypt since 700 AD. The Arabs introduced this species to Spain, where it has been cultivated since 1150. Marco Polo's expeditions also brought the lemon to China in 1297. In addition, it was one of the first new species that Christopher Columbus introduced as seeds to the North American continent, in 1493. In the 19th century, world commercial production of *C. limon* began in Florida and California. Spain is the second largest lemon producer and world leader in fresh exports, occupying second place in the ranking of processing countries[17].

4 Pharmacopeia monographs and its safe use

An essential oil is obtained through the cold-pressing technique of the fresh external parts of the pericarp of *C. limon*: lemon oil (lat. *Citrus limon aetheroleum*, *Limonis aetheroleum*, *Oleum Citri*). This substance is colourless or yellow and has a characteristic and strong lemon aroma[21]. It is considered a pharmacopoeial recognized raw material. Its monographs, entitled *'Limonis aetheroleum'*, are present in the European 9[22] and American[23] Pharmacopoeias, and in the Indian Ayurvedic[24].

Another pharmacopoeial raw material obtained from *C. limon* is the outer part of the mesocarp (the flavedo). Its monograph, entitled *'Citrus limon flavedo'*, can be found in older editions of the French Pharmacopoeia[25].

C. limon also has a positive recommendation in the European Commission Cosmetic Ingredient Database (CosIng Database) as a valuable plant for the production of cosmetic products[26].

The European Food Safety Agency (EFSA) classified *C. limon* fruit and leaves as raw materials of plant origin, presenting natural ingredients that can pose a threat to human health when used in the production of food and dietary supplements. The EFSA has indicated that the toxic substances in these raw materials are photosensitive compounds belonging to the furanocoumarin group, including bergaptene and oxypucedanine (Figure 1)[27].

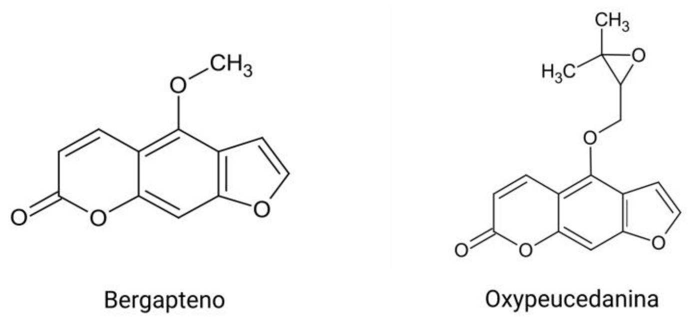

Figure 1. Chemical structure of selected lineal furanocoumarins, which determines the toxic effect of *C. limon.*

C limon essential oil and extracts are classified as safe products on the American Food and Drug Authority (FDA)[28].

5 Chemical composition of *C. limon*

The chemical composition of the *C. limon* fruit has been widely studied. It has been determined, not only for the whole fruit, but also separately for the pericarp, juice, pomace and essential oil. The substances that make up the leaves and the oil extracted from the seeds of *C. limon* are also known. Due to the large number of varieties, crops and hybrids of *C. limon*, various research centres are in charge of analysing the differences in the chemical composition of the raw materials obtained from them.

The most important group of bioactive compounds, both in the fruit of *C. limon* and in its juice, are flavonoids, among which the following predominate: flavonones (eriodictyol), hesperidin, hesperetin, naringin; flavones (apigenin, diosmin); flavonols (quercetin); and its derivatives (Figure 2). Other compounds of this family are also detected in the whole fruit: flavonols–limocitrin (Figure 2) and spinacetin–and flavones–orientin and vitexin (Tables 3 and 4) –. Some flavonoids, such as neohesperidin, naringin, and hesperidin (Figure 2), are characteristic of the *C. limon* fruit. Lemon has the highest eriocitrin content when compared to other *Citrus* species (Figure 2)[29].

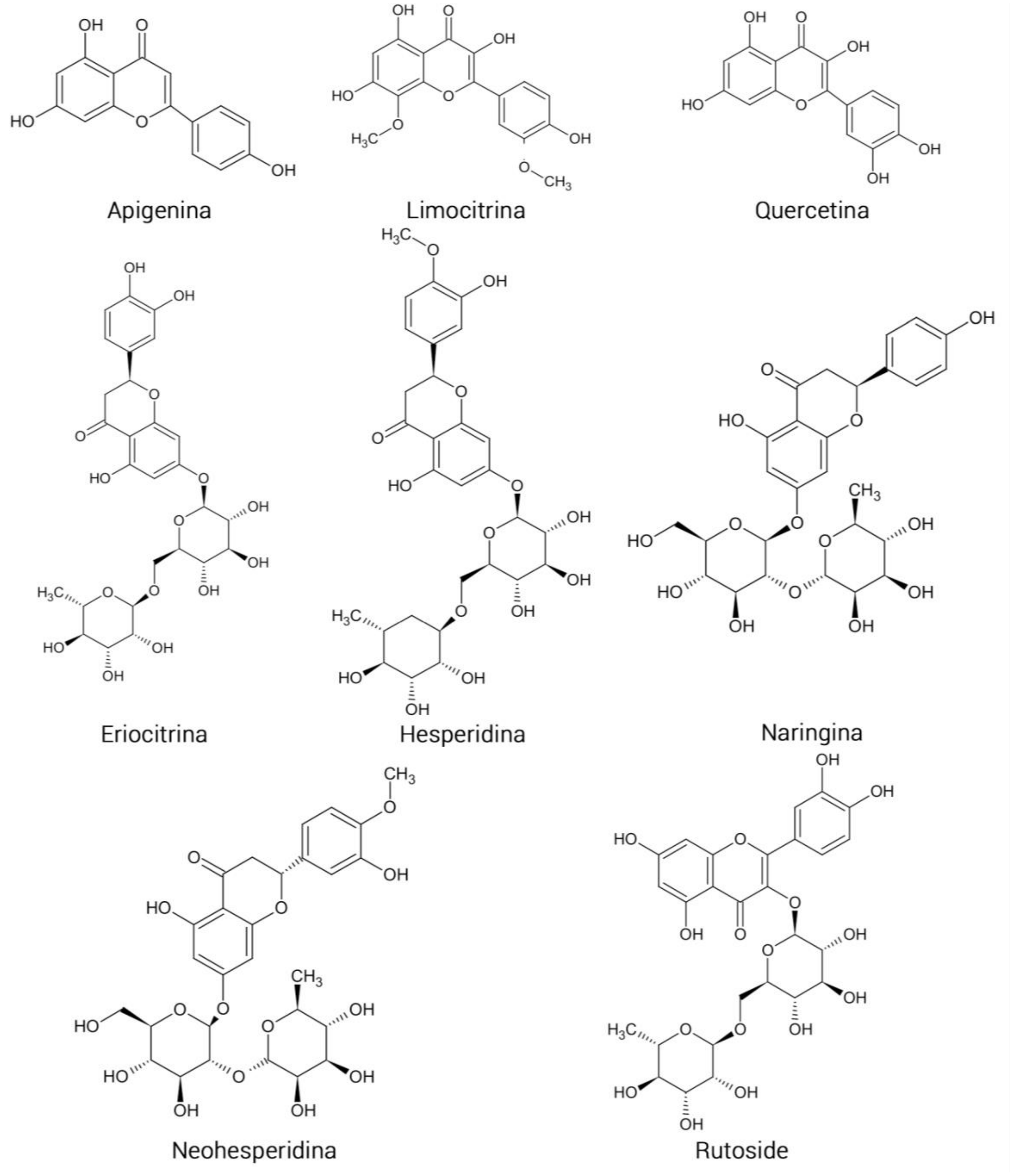

Figure 2. Chemical structure of flavonoids characteristic of *C. limon.*

Another important group of compounds present both in the juice and the fruit is phenolic acids. Ferulic acid and synapic acid stand out, in addition to their derivatives. Moreover, the presence of p-hydroxybenzoic acid has been confirmed, as well as coumarin derivatives, carboxylic acids, amino acid carbohydrates, a complex of B vitamins and, more importantly, Vitamin C (ascorbic acid) in the fruit (Tables 3 and 4)[1,12,13,30–35].

Group of Compounds	Part of fruit	Metabolites
Flavonoids	Whole fruit	Flavonones: eriocitrin, eriodiktyol, hesperidin, naringin, neoeriocitrin, neohesperidin
		Flavones: apigenin, diosmetin, diosmin, homoorientin, luteolin, orientin, vitexin
		Flavonols: isoramnethin, quercetin, limocitrin, rutoside, spinacetin
Limonoids	Whole fruit	Limonin, nomilin
Phenolic acids	Whole fruit	Dihydroferulic acid, p-hydroxybenzoic acid, 3-(2-hydroxy-4-methoxyphenyl) propanoic acid, synapic acid
Carboxylic acids	Whole fruit	Citric acid, galacturonic acid, glucuronic acid, glutaric acid, homocitric acid, 3-hydroxymethylglutaric acid, isocitric acid, malic acid, quinic acid
Coumarins	Whole fruit	Citropten (5,7-dimetoxicumarin), scopoletin
Furanocoumarins	Whole fruit	Bergamottin
Amino acids	Whole fruit	L-alanine, L-arginine, L-asparagine, L-aspartic acid, dimethylglycine, glutamic acid, L-phenylalanine, DL-proline, L-tryptophan, L-tyrosine, L-valine
Carbohydrates	Fruit peel	Monosaccharides: arabinose, fructose, β-fructofuranose, β-fructopyranose, galactose, glucose, mannose, myoinositol, rhamnose, scylloinositol, xylose
	Whole fruit	Disaccharides: sucrose
Vitamins and metabolites	Whole fruit	Choline, pantothenic acid, trigoneline, Vitamin C
Macroelements	Fruit peel and pulp	Calcium (Ca), magnesium (Mg), phosphorus (P), potassium (K), sodium (Na)

Table 3. Composition of *C. limon* fruit extract.

Compound Group	Metabolites
Flavonoids	Flavonones: hesperidin, naringin Flavones: apigenin, chrysoeriol, diosmetin, luteolin Flavonols: isoramnethin, quercetin, rutoside dihydroxyflavonols: dihydroxyisoramnethin-7-O-rutinoside
Phenolic acids	Ferulic acid, synapic acid
Vitamins	Vitamins: C (53 mg/L), A, B1, B2, B3

Table 4. Composition of *C. limon* fruit juice.

Another interesting group of compounds present in the *C. limon* fruit are the limonoids. These substances are highly oxidized secondary metabolites with polycyclic triterpenoid skeletons. Inside the fruit, it predominates in the seeds, the pulp and the rind. Two compounds of this type stand out mainly: limonin and nomilin (Figure 3)[36]. Studies have shown that the concentrations of the compounds in this group depend on the growth and ripening stages of the fruit. Young citrus fruits have a higher content of these molecules than ripe ones[37].

The analysis of the macroelements present in *C. limon* fruit showed the presence in pulp and peel of: calcium (Ca), magnesium (Mg), phosphorus (P), potassium (K) and sodium (Na)[35].

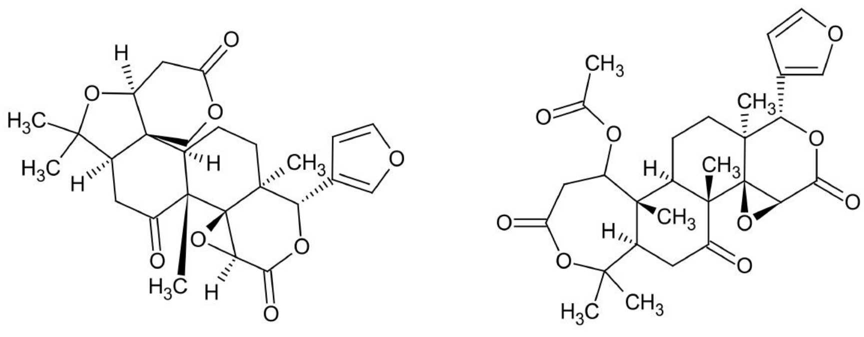

Figure 3. Chemical structure of limonoids characteristic of *C. limon.*

In *C. limon* seed oil, fatty acids are the main ingredients, such as arachidonic acid, behenic acid and linoleic acid, as well as tocopherols and carotenoids (Table 5)[32,34]. Recent studies have shown that the oil from the pulp's fruit contains more fatty acids compared to other citrus species, such as *C. aurantium, C. reticulata* and *C. sinensis*. Among them, the following have been identified: behenic acid, erucic acid, godoic acid, lauric acid, linoleic acid, α-linolenic acid, margaric acid, palmitic acid, palmitoleic acid, pentadecanoic acid and stearic acid[39].

Compound Group	Metabolites
Fatty acids	Arachidonic acid, behenic acid, lignoceric acid, linoleic acid, linolenic acid, oleic acid, palmitoleic acid, palmitic acid, stearic acid
Tocopheroles	α-tocopherol, β-tocopherol, γ-tocopherol, δ-tocopherol
Carotenoids	β-carotene, β-cryptoxanthin, lutein

Table 5. Composition of oil from *C. limon* seeds.

Monoterpenoids are the main components of lemon pericarp essential oil. Among them, the following predominate quantitatively: limonene (69.9%), β-pinene (11.2%), γ-terpinene (8.21%), (Figure 4), sabinene (3.9%), myrcene (3 , 1%), geranial (E-citral, 2.9%), neral (Z-citral, 1.5%), linalool (1.41%). In addition to terpenoids, the essential oil also contains linear furanocoumarins (psoralens) and polymethoxylated flavones (Table 6)[14,40,41]. The UNE-ISO

855 standard specifies certain characteristics of lemon essential oil, as well as the chromatographic profile of the main components present in lemon essential oil[91].

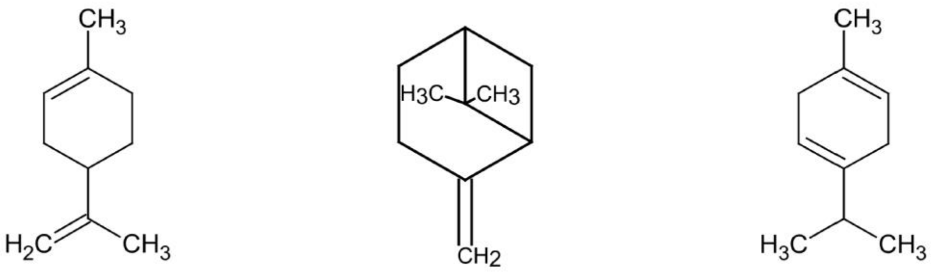

Figure 4. Chemical structure of selected terpenoides characteristics of *C. limon* essential oil.

Group of Compounds	Essential Oil	Metabolites
Terpenoids	Essential oil of the *C. limon* pericarp	Limonene (69.9%), p-mentha-3,8-diene (18.0%), β-pinene (11.2%), γ-terpinene (8.21%), myrcene (4.4%), sabinene (3.9%), myrcene (3.1%) geranial (E-citral, 2.9%), neral (Z-citral, 1.5%), linalool (1.41%), α-pinene (1.1%), α-thujene (1.1%), β-bisabolene (0.5%) (E)-β-ocimene (0.4%), citronellol (0.3%), geraniol (0.2%), β-caryophyllene (0.2%), trans-muurala-4(14),5-diene (0.2%), α-terpinene (0.1%), terpinolene (0.1%), nonanal (0.1%), eucalyptol (0.1%); other terpenes below 0.06%: α-bisabolol, camphene, citronellal, citronellyl acetate, β-curcumene, γ-curcumene, p-cymene, 7-epi-sesquithujene, α-farnesene, α-felandren, cis-limonene, trans-limonene, octanal, octanal acetate, terpinen-4-ol, β-santalene, zonarene
	Essential oil of the *C. limon* leaf	Limonene (31.5%), sabinene (15.9%), citronellal (11.6%), linalool (4.6%), neral (4.5%), geranial (4.5%), (E)-β-ocimene (3.9%), myrcene (2.9%), citronellol (2.3%), β-caryophyllene (1.7%), terpne-4-ol (1.4%), geraniol (1.3%), α-pinene (1.2%),γ-terpinene (0.9%), sylvestrene (0.6%), α-terpineol (0.6%), isogeranial (0.4%), β-bisabolene (0.3%), germacrene B (0.3%), isospathulenol (0.3%), α-terpinene (0.2%), terpinolene (0.2%), isopulegol (0.2%), γ-terpineol (0.2%), decanal (0.2%), δ-elemene (0.2%), α-humulene (0.2%), α-cadinol (0.2%), *epi*-α-bisabolol (0.2%) *cis*-p-menth-2-en-1-ol (0.1%), isoneral (0.1%), γ-muurolene (0.1%), spathulenol (0.1%)
Furanocoumarins	Essential oil of the *C. limon* pericarp	Aprindine, bergamottin, bergapten, byakangelicol, byakangelicin, epoxybergamottin, 5- and 8-geranoxypsoralen, 8-geranyloxypsoralen, heraclenin, imperatorin, isoimperatorin, isopimpinellin, xanthotoxin, oxypucedanin, phellopterin, psoralen
Cumarinas	Essential oil of the *C. limon* pericarp	Citropten, 5-geranyloxy-7-methoxycoumarin, herniarin, 5-isopentenyloxy-7-methoxycoumarin

Table 6. Chemical composition of the essential oil of the *C.* limon pericarp and leaf.

C. limon's leaf essential oil differs in composition from the pericarp oil. Its main compounds are: limonene (31.5%), sabinene (15.9%), citronellal (11.6%), linalool (4.6%), neral (4.5%), geranial (4.5 %), (E) -β-ocimene (3.9%), myrcene (2.9%), citronellol (2.3%), β-caryophyllene (1.7%), terpne-4-ol (1 , 4%), geraniol (1.3%) and α-pinene (1.2%) (Table 6)[14,16,40–43].

6 Studies of the metabolic profile

The research team of the Department of Chemical and Geological Sciences of the University of Modena (Italy) studied the metabolic profile of different parts of the *C. limon* fruit such as the flavedo, albedo, pulp, oil glands and the seeds of the fruit using High-resolution magic-angle spinning nuclear magnetic resonance spectroscopy (HR-MAS NMR)[34]. The analyses were performed directly on intact tissues with no physicochemical manipulation.

In *C. limon* flavedo, the following compounds were detected: Terpenoids (limonene, β-pinene and γ-terpinene), amino acids (asparagine, arginine, glutamine, proline), organic acids (malic acid and quinic acid), osmolites (stachidrine), and sugars (glucose, fructose, β-fructofuranose, myoinositol, scylloinositol and sucrose) (Table 3).

The presence of small amounts of amino acids (alanine, threonine, valine, glutamine), sugars (glucose, sucrose, β-fructofuranose, myoinosyltin, scenoinositol and β-fructopyranose), and osmolytes (stachidrin, β-hydroxybutyrate, ethanol) has been demonstrated in the albedo of the *C. limon* fruit (Table 3). Furthermore, flavonoids such as hesperidin and rutoside have been identified by High Performance Liquid Chromatography (HPLC). The HR-MAS NMR analysis of the oil glands composition showed the presence of terpenoids (limonene, γ-terpinene, β-pinene, α-pinene, geranial, neral, citronellal, myrcene, sabinene, α-thujene, nerol and geraniol esters) and sugars (glucose, sucrose, β-fructofuranose and β-fructopyranose). Amino acids (asparagine, proline, alanine, γ-aminobutyric acid (GABA), glutamine, threonine and valine), organic acids (citric acid and malic acid), sugars (glucose, sucrose, β-fructofuranose, β-fructopyranose, myoinosytol and escicolloinosytol) and osmolytes (stachidrine, ethanol and methanol) have been identified in the pulp (Table 3). The analysis of the seeds by HR-MAS NMR indicated that triglycerides (linoleic acid, linolenic acid and their derivatives), sugars (glucose and sucrose), osmolytes (stachidrine) and trigonelline are predominant in their composition[34].

In another metabolomic study, extracts from the peel of ripe *C. limon* fruit were characterized. It contains non-fluorescent chlorophyll catabolites (NCCs) and non-fluorescent chlorophyll catabolites of the dioxobilane type (DNCC). Four different NCCs were detected in the *C. limon* fruit peels: Cl-NCC_1, Cl-NCC_2, Cl-NCC_3 and Cl-NCC_4[43].

On the other hand, it has been revealed the presence in the lemon leaf of 26 different organic acids and their derivatives (aconitic acid, 2-aminobutyric acid, 4-aminobutyric acid, ascorbic acid, benzoic acid, citric acid, p-coumaric acid, Ferulic Acid, Fumaric Acid, Glycolic Acid, 3-Hydroxybutyric Acid, 2-Isopropylalic Acid, Malic Acid, Malonic Acid, 3-Methylglutaric

Acid, Oxamic Acid, D-3-Phenylacetic Acid, Pipecolic Acid, Pyruvic Acid, Quinic Acid, Acid shichemical, succinic acid, threonic acid, uraponic acid), 21 amino acids (alanine, γ-aminobutyric acid, anthranilic acid, asparagine, aspartic acid, glutamic acid, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline , pyroglutamic acid, serine, threonine, tryptophan, tyrosine, valine) and 13 sugars and sugar alcohols (arabinose, fructose, galactose, glucose, glycerol, inositol, lixose, maltose, rhamnose, ribose, sorbose, sucrose, xylitol). Furthermore, when the studied leaves were exposed to stress conditions induced by jasmonic and salicylic acid, the amino acid content increased[44].

The profile of volatile and non-volatile metabolites in the *C. limon* essential oil depends on the geographical origin and the analytical methods used. For example, the essential oil of the Italian lemon fruit is enriched in α-thuyen, α-pinene, α-terpinene, sesquiterpenoids (β-caryophyllene) and furocoumarins (bergamotin). On the other hand, the essential oil of the fruits of the Spanish and Argentine plants, present a significant proportion of terpenes such as limonene, while they present differences at the level of imperatorine and biacangelicol. In the case of Spanish fruit, there is a greater amount of camphor and 4-534pineol, whilst the Argentinian has more sabinene and cis-sabinene hydrate[45].

Research focused on the identification of components in the essential oil of different citrus species, including *C. limon*, showed that in general, most of the essential oil components studied were identified as monoterpenoids. For the particular case of lemon, the following were found: limonene (70.37%), p-mentha-3,8-deno (18.00%), myrcene (4.40%), α-pinene (3.24%), α-thuyen (1.05%) and terpinolene (0.90%) (Table 6). To a lesser extent, the following were found: sabinene (0.28%), α-terpinene (0.22%), trans-muurola-4 (14), 5-diene (0.18%), eucalyptol (0.12%), octanol acetate (0.03%), β-turmeric (0.03%), zonarene (0.03%), 7-epi-sesquitujen (0.02%), citronellyl acetate (0.02%) oα-farnesene (0.01%) (Table 6). The metabolic profile model shown can be used to clearly discriminate the basic *Citrus* species. Limonene, α-pinene, sabinene and α-terpinene were the main characteristic components of the analysed metabolomes of the *Citrus* genotypes that contributed to their taxonomy[16].

Another study in *C. limon* has shown that the essential oil contains high amounts of furanocoumarins and coumarins compared to other essential oils of citrus. Paticularly, 13 furanocoumarins have been detected (bergamotin, bergaptene, byakangelycol, byakangelicin, epoxybergamotin, 8-geraniyloxypsoralen, heraclenin, imperatorin, isoimperatorin, isopimpinellin, oxipeucedanine, oxipeucedanine hydrate, felopterin) and two coumarins (citropten and herniarin) (Table 6)[42].

7 Functionality and biological effectiveness of *C. limon*

7.1 Anticancer activity

Various *in vitro* tests sustain the ability of the compounds contained in lemon to destroy cancer cells. A study using isolated lemon juice nanovesicles showed that they inhibited the proliferative capacity of various tumour cell lines by activating apoptosis mediated by the TRAIL ligand. Furthermore, its effect *in vivo* has also been demonstrated in models of chronic myeloid leukemia (CML) through its specific action on tumour cells and the activation of the same TRAIL-dependent apoptotic pathways (Table 7)[46]. Another work showed that an extract of lemon seeds showed inhibitory capacity for cell proliferation in the MCF-7 breast adenocarcinoma model cell line. The results of this trial suggested that both the aglycones and the limonoid and flavonoid glucosides present in the extract could serve as agents to prevent breast cancer (Table 7)[9].

7.2 Antioxidant activity

The antioxidant activity of *C. limon* flavonoids (hesperidin and hesperetin) has been demonstrated to not only be limited to their activity to scavenge free radicals, but also increase antioxidant cellular defences through the ERK/Nrf2 signalling pathway (Table 7)[8].

7.3 Anti-inflammatory activity

Several *in vitro* and *in vivo* studies have been developed to evaluate the efficacy of hesperidin metabolites or its synthetic derivatives in reducing inflammation mediators such as NFκB, iNOS or COX2, as well as chronic inflammation markers (Table 7)[8]. Furthermore, lemon essential oil has shown anti-inflammatory effects in different animal models, comparable to those obtained with pure D-limonene. The authors of this work propose that the anti-inflammatory effect of essential oil would be mediated by the high concentration of this present compound (Table 8) [48]. According to these findings, the antioxidant potential of D-limonene has been revealed in an animal model of Crohn's disease, in which subjects treated with this compound saw intestinal inflammation significantly reduced at an anatomical, histological level and regarding the levels of the pro-inflammatory cytokine TNFα49. On the other hand, in an experimental model of ischemia-reperfusion, a hepatoprotective effect of limonin has been reported, related to its antioxidant and anti-inflammatory activity, through the inhibition of signalling by Toll-type receptors (TLR)[50]. In an *in vitro* enzyme activity study, the inhibitory capacity of the enzyme 5-lipoxygenase (5-LOX) has been demonstrated by oil extracted from the skin of pink or variegated lemon. The authors of this study attribute this activity, directly linked to inflammatory processes, to the high D-limonene content of this oil[51].

7.4 Antimicrobial activity

7.4.1 Antibacterial activity

The antimicrobial activity of different lemon extracts has been evaluated in a wide variety of scientific works. Otang and Afolayan recently published that extracts of lemon peel made with organic solvents showed inhibitory capacity for the growth of various microorganisms. Among the Gram-negative bacteria, *Salmonella typhimurium* and *Shigella sonnei* stood out, while the most sensitive Gram-positive bacteria were *Enterococcus faecalis* and *Bacillus subtilis*. (Table 7)[7].

In other studies, essential oils from lemon peel also showed inhibition of the growth of Gram-positive bacteria (*Bacillus subtilis, Staphylococcus capitis* and *Micrococcus luteus*) and Gram-negative bacteria (*Pseudomonas fluorescens, Escherichia coli*) in different experimental systems[51,52].

It is worth mentioning the potential of *C. limon* essential oil against caries-causing bacteria, such as *Streptococcus mutans*. A team of researchers from the Tianjin Medical University (China) developed a study that showed that lemon essential oil reduced the growth of *S. mutans*, as well as its ability to adhere to surfaces and the expression of the enzyme glucosyl transferase, the latter being essential for the formation of bacterial biofilms responsible for dental caries (Table 8)[53].

7.4.2 Antifungal activity

In addition to antibacterial activity, organic solvent extracts from lemon peel also have antifungal activity against *Candida glabrata*, one of the organisms responsible for superficial cutaneous mycoses (Table 7)[7]. Monoterpenes of this type of extracts have been associated with activity against *Aspergillus niger, Saccharomyces cerevisiae* and *Candida parapsilosis* (Table 8)[51,53]. Another study confirmed that *C. limon* essential oil inhibits the growth of *C. albicans*[55].

Activity	Mechanism of Action	Ref.
Anticancer activity	Inhibition of the proliferation of cancer cells. Activation of "TRAIL"-apoptopic cell death. Inhibition of tumour growth in chronic yelogenous leukaemia (CML) Antioxidant action and induction of apoptosis in MCF-7 cells (breast adenocarcinoma cells) (*C. limon* seed extract).	9, 46
Antioxidant activity	Augmention of antioxidant cellular defences via ERK/Nrf2 signalling pathway.	8, 47
Anti-inflammatory activity	Inhibition of NF-κB factor, nitric oxide (II) synthase (iNOS), induced cyclooxygenase (COX-2) (hesperidin, hesperitin). Down-regulation of TLR-signaling pathway (limonin).	8, 48, 49, 51
Antibacterial activity	Inhibiting activity against Gram-positive bacteria: *Enterococcus faecalis*, *Bacillus subtilis.* Inhibiting activity against Gram-negative bacteria: *Salmonella typhimurium*, *Shigella sonnei*, *Helicobacter pylori.*	5, 7
Antifungal activity	Inhibiting activity against *Candida glabrata* strains.	7
Antiviral activity	Inhibition of replication of *Herpes simplex.*	56
Anti-allergic activity	Inhibition of histamine secretion in peritoneal cells of rats.	1, 58
Hepatoregenerative activity	Normalization of alanine aminotransferase (ALAT), alkaline phosphatase (ALP) and bilirubin. Reduction in malonic dialdehyde (MDA), lipid peroxidation, superoxide dismutase (SOD) and catalase.	10
Prevention of diabetes	Inhibition of gluconeogenesis (naringenin, hesperitin) Reducing wound-healing time. Increasing tissue growth rate, collagen synthesis, and protein and hydroxyproline concentration.	1, 59
Dietary activity	Lowering blood lipids. Reducing the levels of insulin, leptin and adiponctin in the blood.	1, 61
Effects on the cardiovascular system	Improvement of blood parameters in detoxifying diet. Decreasing blood fibrinogen. Limiting myocardial damage (naringenin).	1, 11, 62
Effects on the nervous system	Strengthening short-term memory. Ansiolitic effect.	66
Effects on the skeletal system	Decreasing TRAP-positive multinucleated cell numbers (nomilin). Decreasing bone resorption activity (nomilin). Down regulation osteoclast-specific genes (nomilin).	65, 68

Table 7. Biological activity of *C. limon* extract.

7.4.3 Antiviral activity

In addition to the antibacterial and antifungal effects, there are *in vitro* studies in which the ability of lemon essential oils to inhibit the replication capacity of the Herpes Simplex virus 1 (HSV-1), which causes cold sores, has been detected[56].

7.5 Anti-parasite effect

The effect of *C. limon* essential oil on *Sarcoptes scabiei* var. *Cuniculi* (itch mite) has been evaluated both *in vitro* and *in vivo*. The results of the *in vitro* application showed that this substance caused mortality in 100% of the mites after 24 h. The *in vivo* administration of lemon oil in naturally infected rabbits showed a complete recovery of the clinical signs and the absence of mites in the microscopic examination from the second week of treatment (Table 8)[57].

7.6 Anti-allergic effect

Aqueous extracts from the *C. limon* rind have been used to study their effects on histamine release. In an experimental model in which the release of this substance was induced in rat peritoneal exudate, it was demonstrated that these extracts inhibited the process. Furthermore, they were potential suppressors of inflammation of the mice's peritoneal cavity at levels comparable to indomethacin, a well-characterized anti-inflammatory compound (Table 7)[58].

7.7 Hepatic effects

The hepatoprotective and hepatoregenerative capacity of organic extracts of lemon fruit has been evaluated both *in vivo* and *in vitro*. In the first case, experimental animals that were induced liver damage with carbon tetrachloride (CCl_4) and subsequently treated with lemon extract experienced a normalization of the levels of transaminases, alkaline phosphatase and bilirubin, and a decrease in oxidative stress in the liver, versus untreated animals. On the other hand, the cytotoxicity generated by CCl_4 *in vitro* in the human hepatic cell line HepG2 was reversed in a dose-dependent manner when applying these extracts[10]. Other studies with *C. limon* essential oil have also shown the stimulation of liver detoxification through the activation of cytochrome P450 and liver enzymes (glutathione S-transferase) in chronic liver poisoning (Table 8)[21].

7.8 Antidiabetic effect

The protective effect against diabetes of the compounds contained in lemon has been contrasted in animal models of diabetes induced by streptozotocin. On the one hand, extracts obtained from lemon peel were able to lower blood glucose, reduce wound healing time, and improve skin matrix protein synthesis, processes that are altered in this pathology (Table 7)[60]. On the other hand, in this same animal model, the administration of pure D-limonene resulted in a decrease in blood glucose, the proportion of glycated haemoglobin, the activity of gluconeogenic enzymes, as well as an increase in the activity of glucokinase and the amount of hepatic glycogen, compared to diabetic individuals in the absence of treatment (Table 8)[61].

7.9 Dietary applications

Studies have shown that D-limonene is beneficial in reversing dyslipidaemia and hyperglycaemia *in vivo*. In animal models, its administration promotes a decrease in LDL cholesterol, prevents the accumulation of lipids and regulates blood sugar levels. In addition, these effects are enhanced by its antioxidant activity. On the other hand, dietary supplementation with D-limonene would restore the pathological alteration of the liver and pancreas, thus being able to help in the prevention of obesity (Table 8)[21].

7.10 Effects on the digestive system

Various studies have shown that D-limonene increases gastric motility and causes a reduction in nausea, neutralization of stomach acids and relief of gastric reflux (Table 8)[21].

Moreover, *in vitro* studies sustain that the application of limonene on human intestinal cells favours the barrier function of the intestinal epithelium, a highly relevant aspect for the regulation of the passage of nutrients to the bloodstream and for the immune system's component activity[50].

7.11 Effects on the cardiovascular system

A study that addressed the use of lemon juice as a dietary supplement in a low-calorie intake regimen showed that this component caused a reduction in high sensitivity C-reactive protein in serum (hs-CRP) compared to placebo and the normal diet group. Haemoglobin and haematocrit levels remained stable in the group on the lemon detox diet, while they decreased in the placebo and normal diet groups (Table 7)[61].

Additionally, lemon juice has also shown positive effects regarding coagulation parameters. *In vitro* studies have confirmed that this substance increases the time that different pro-coagulant enzymes, such as thrombin and thromboplastin, use to exert their activity. Moreover, in this same experimental system, lemon juice reduced the concentration of fibrinogen, a protein precursor necessary for the formation of clots. Besides, the administration of lemon juice to experimental animals increased the levels of red blood cells and haemoglobin and showed increases in the bleeding time before a wound and the reaction time of thrombin. Together, these results support an anti-coagulant activity of lemon juice (Table 7)[11].

The effect of lemon components has also been tested in models of major cardiovascular diseases in the Western world, such as myocardial infarctions. The administration of naringenin in an animal model of ischemia-reperfusion in myocardial tissue has shown improvements in heart function and a reduction in infarction and cell death in myocardial tissue compared to untreated animals[64].

7.12 Activity in the nervous system

C. limon juice influence on the memory of mice has been researched using observational experiments on the behaviour of animals. Passive avoidance is an animal behaviour used to evaluate the short or long-term memory of small animals, which measures the latency when entering a black compartment. Animals that were fed *C. limon* juice showed a highly significant or significant increase in latency before entering a black compartment after 3 and 24 h, respectively (Table 7)[62]. These results would sustain that lemon juice favours short-term memory in experimental animals.

Studies have also shown that the main compound in *C. limon* essential oil, D-limonene, administered by inhalation to mice, has a significant calming and anxiolytic effect by activating serotonin and dopamine receptors. Furthermore, D-limonene has an inhibitory effect on pain receptors similar to that of indomethacin and hyoscine, two well-known and characterized analgesics (Table 8)[63].

7.13 Activity in the skeletal system

Studies have shown the potential use of nomilin, one of the compounds present in the *C. limon* fruit (Table 3) for the inhibition of bone resorption in an *in vitro* approach. Nomilin has shown a significant reduction in the differentiation of macrophages to osteoclasts. On the other hand, non-toxic concentrations of this compound decreased the resorption capacity of bone and genes involved in the specific regulation of osteoclasts. This study supports the therapeutic potential of nomilin for the prevention of metabolic bone diseases such as osteoporosis[65].

Activity	Mechanism of Action	Ref.
Anticancer activity	Stimulation of apoptosis of colorectal cancer cells.	69
Anti-inflammatory activity	Inhibiting cell migration. Inhibition of cytokine production. Inhibition of inflammation mediator (D-limonene). Inhibition of leukocyte chemotaxis (D-limonene). Interaction with 5-lipoxygenase, TNF-α (tumour necrosis factor), IL-6 (interleukin-6).	48, 51
Antibacterial activity	Inhibiting activity against Gram-positive bacteria: *Staphylococcus capitis*, *Micrococcus luteus*, *Bacillus subtilis*. Inhibiting activity against Gram-negative bacteria: *Pseudomonas fluorescens*, *Escherichia coli*.	21, 51, 52
Anticaries activity	Inhibiting growth of *Streptococcus mutans*, its adhesión property and GST expression.	53
Antifungal activity	Inhibiting activity against: *Aspergillus niger*, *Saccharomyces cerevisiae*, *Candida parapsilosis* strains (monoterpenes).	
Antiviral activity	Inhibition of the virus *Herpes simplex*.	56
Antiparasitic activity	Treatment of schistosomiasis caused by Schistosoma mansoni (D-limonene). Inhibitory effect on *Sarcoptes scabiei* development.	57
Hepatoprotective and detoxification activity	Stimulation of liver detoxification by activation of cytochrome P_{450} and liver enzymes (glutathione S-transferase) in chronic liver poisoning.	70
Diabetes prevention	Decreased glycolized haemoglobin (D-limonene). Decreased gluconeogenesis enzymes: glucose-6-phosphatase and fructose-1,6-biphosphatase (D-limonene). Decreased blood glucose (D-limonene).	60
Anti-obesity activity	Lowering cholesterol and preventing fat deposits (D-limonene). Equalization of blood sugar (D-limonene). Regeneration of pathological changes in the liver and pancreas.	71
Effects on the digestive system	Increased gastric motility and reduction of nausea (D-limonene). Neutralization of stomach acids (D-limonene). Relief of gastric reflux (D-limonene). Increased bile flow.	21
Lipolytic and cholesterol-lowering activity	Reducing the level of triglycerides, LDL and increasing the level of HDL cholesterol in the blood. Lowering cholesterol and arachidonic acid levels by stimulating liver enzymes and cytochromes. Lipolytic effect (γ-terpinene and p-cymene).	1, 21, 71
Effects on the nervous system	Inhibitory effect on pain receptors similar to that of indomethacin and hyoscine (D-limonene). Sedative and anxiolytic effect by activating serotonin and dopamine receptors.	72

Table 8. Biological activity of *C. limon* essential oil confirmd by scientific research.

7.14 *C. limon* in pharmacy

Besides the important applications mentioned above, lemon essential oil is used in pharmaceutical and cosmetic formulations as a flavour and aroma corrector, as well as a natural preservative, due to its confirmed antibacterial and fungistatic effects[21].

8 *C. limon* in the food industry

Due to the rich chemical composition of the *C. limon* fruit and other raw materials derived from lemon, the applications in the food industry and in food processing is very wide. The lemon fruit is mainly used as fresh fruit, but it is also processed to make juices, jams, jellies, molasses, etc.[40]. Fresh lemon fruit remains fresh for several months, maintaining its levels of juice, vitamins, minerals, fibre, and carbohydrates. During storage and industrial processing, the content of Vitamin C (ascorbic acid) in lemon fruits and juices decreases. The factors responsible for this reduction are oxygen, heat, light, storage time, temperature and storage duration. In order to avoid the reduction of ascorbic acid levels and the antioxidant capacity of both lemon fruit and lemon juice, they must be kept at 0-5 ºC and protected from water loss by means of adequate packaging, with high relative humidity during its distribution. Under such conditions, lemon products show good retention of Vitamin C and antioxidant capacity[40,73].

C. limon fruit rind is rich in pectin, which is used in a wide range of industrial food processes as a gelling agent, including the production of jams and jellies, and as a thickener, texturizer, emulsifier and stabilizer in dairy products. Due to its gelatinizing properties, pectin is also used in pharmaceutical, dental and cosmetic formulations[74].

Lemon juice is used as an ingredient in beverages, particularly lemonade and sodas, and in other foods, such as salad dressings, sauces, and baked goods. Lemon juice is a natural flavouring and preservative and is also used to add an acidic or bitter taste to food and soft drinks[40,75].

C. limon juice is the most suitable, since it is pesticide residues free, to improve the taste of liqueurs, for example, "limoncello", the traditional Sicilian liqueur. This beverage is made by macerating the lemon peel in ethanol, water and sugar[40,75].

Currently, two compounds isolated from lemon essential oil, linalol and citral, are mainly used as flavourings and natural preservatives due to their functional properties (antimicrobial, antifungal, etc.)[51,2]. In particular, they are often used to extend the short shelf life of seafood and in the production of some types of cheese since they significantly reduce microorganism population, especially those of the *Enterobacteriaceae* family[40,75].

9 Applications in the cosmetic industry

The extracts of the *C. limon* fruit and its essential oil, as well as the active compounds isolated from these raw materials, have become the subject of numerous scientific studies aimed at testing the possibility of their use in cosmetology. Lemon products have long shown a positive effect on acne-prone skin that is easily affected by sunburn or fungal infections. In this sense, the traditional uses of these raw materials are known in various parts of the world. In Tanzania, *C. limon* fruit juice is mixed with egg albumin, honey, and cucumber, and applied to the skin every day at night to smooth facial skin and treat acne[76]. The freshly squeezed fruit juice of *C. limon* mixed with olive oil is used as a natural remedy for the treatment of hair and scalp disorders in the West Bank, Palestine77. Currently, the knowledge on the cosmetic activity of *C. limon* is in constant expansion.

The essential oil of *C. limon* shows antibiotic and flavoring properties, and for this reason it is used in formulations of shampoos, toothpaste, disinfectants, topical ointments and other cosmetic products[40].

Scientific studies have shown a significant antioxidant effect of *C. limon* fruit extracts, which is why its use is recommended in anti-aging cosmetics[8,47]. The use of different vectors of *C. limon* extracts (e.g. hyalurosomes, glyerosomes) in cosmetic production technology contributes to even greater inhibition of oxidative stress in crucial skin components, including keratinocytes and fibroblasts (Table 9)[78]. In addition, *C. limon* Vitamin C is used as an ingredient in specialized dermo-cosmetics. Its exogenous application increases collagen production, which softens and tightens the skin. It is used in anti-aging products, to reduce superficial wrinkles, and as an antioxidant with a synergistic effect when combined with vitamin E[47].

C. limon essential oil ingredients (including citral, β-pinene, D-limonene) have a depigmenting effect mediated by their tyrosinase enzyme's inhibitory activity [79]. Moreover, essential oil has been shown to promote lipid penetration, as well as water-soluble vitamin penetration. Therefore, it can be used as a promoter of active substance penetration through the skin[80]. In addition to the direct effect on the skin, the essential oil can also be used as a natural preservative and as a concealer in cosmetic products. Different studies have confirmed its antibacterial and fungistatic effects (Table 9)[7,51,52].

Activity	Extracts and Coumpounds Tested	Mechanism of Action
Antioxidant activity	*C. limon* essential oil	Strong lipid peroxidation reduction and free radical reduction effect *in vitro* and *in vivo*[78-79].
	C. limon var. *pompia* fruit extracts	Extract enclosed in hyalurosomes and glycerosomes reduces oxidative stress caused by hydrogen peroxide and the viability of keratinocytes and fibroblasts[79].
Depigmentating activity	Essential oil ingredients (e.g., citral, β-pinene, D-limonene)	Essential oil components show tyrosinase inhibitory activity. Mixture of essential oil ingredients has a stronger inhibitory effect due to their synergistic effect[80].
Effect of increasing the penetration of substances	*C. limon* essential oil	Increasement of the penetration of α-tocopherol. Improvement of the penetration of locally administered lipids and water-soluble vitamins[81].
Preservative effect in cosmetics	*C. limon* essential oil	Antibacterial activity and increasing the fungistatic effect of synthetic preservatives[7,51,52].

Table 9. Biological activity of *C. limon* fruit extracts, essential oil and its ingredients, significant from the cosmetic industry point of view.

Furthermore, *C. limon* pericarp extract also helps accelerate the regeneration of diabetic wounds. Additionally, the essential oil derived from *C. limon*'s pericarp has shown anti-inflammatory, antiallergic and slimming properties[48,58,59,61].

According to the CosIng database (Database of cosmetic ingredients), *C. limon* can be used in twenty-three different ways: as oils obtained from various organs, in the form of extracts, hydrolates, powdered parts of the plant, wax and juice[26]. The most common activity defined by CosIng for the raw material of this species is to maintain the skin in good condition, improve the odour of cosmetic products and mask the odour of other ingredients in cosmetic preparations[26]. Approved forms of raw materials and their possible effects, as well as their use as correctors, are summarized in table 10[26].

The form	Activity
C. limon (lemon)/*Fucus serratus* extract	Skin conditioning
C. limon bud extract humectant,	Skin conditioning
C. limon flower water humectant, skin conditioning	Skin conditioning
C. limon flower/leaf/stem extract	Masking, skin conditioning, tonic
C. limon flower/leaf/stem	Oil masking
C. limon fruit extract masking, skin conditioning	Masking, skin conditioning
C. limon fruit oil astringent, tonic	Astringent, tonic
C. limon fruit powder	Skin conditioning
C. limon fruit water	Masking, skin conditioning

C. limon juice	Skin conditioning, tonic
C. limon juice extract	Tonic
C. limon juice powder	Skin conditioning, tonic
C. limon leaf extract	Perfuming
C. limon leaf oil	Perfuming, masking
C. limon leaf/peel/stem oil skin conditioning	Skin conditioning
C. limon peel	Masking, skin conditioning
C. limon peel cera/*C. limon* peel wax	Skin conditioning
C. limon peel extract	Emollient, skin conditioning, skin protecting, tonic
C. limon peel oil	Masking, perfuming, skin conditioning
C. limon peel powder	Absorbent, viscosity controlling
C. limon peel water	Skin conditioning
C. limon seed oil	Masking, perfuming, skin conditioning

Table 10. *C. limon* in cosmetic products according to CosIng.

The essential oil of *C. limon* has been used since the 18th century in the production of the famous 'Eau de Cologne'. In aromatherapy, it is used to treat numerous lifestyle-related diseases and ailments: hypertension, neurosis, anxiety, varicose veins, arthritis, rheumatism, and mental heaviness. It also relieves the characteristic symptoms of menopause. As an additional application, it is used in massages to relax muscles and promote deep calm and relaxation[21].

Despite the beneficial effects of the compounds present in lemon, *C. limon* fruit extracts and essential oils should not be used in high concentrations in baths or directly on the skin. Accordingly, recent studies have shown that the D-limonene contained in the oil has an allergenic and irritant effect on the skin. After applying cosmetics containing *C. limon* oil, the skin should not be exposed to sunlight, as it contains photosensitizing compounds belonging to the linear furanocoumarin group. The lemon pericarp contains: bergapten, felopterin, 5- and 8-geranoxypsoralen, and the essential oil contains: bergapten, imperatorin, isopimpinellin, xanthotoxin, oxipeukedanine and psoralen[21,81].

The International Fragrance Association (IFRA) has issued restrictions on the use of *C. limon* essential oil. In preparations that remain on the skin, the concentration of this oil should not exceed 2%. Also, *C. limon* essential oil should not be used in preparations that remain on UV-exposed skin. They should not contain more than 15 ppm of bergaptene[82].

10 Plant biotechnology studies *C. limon*

Plant biotechnology creates opportunities for the potential use of *in vitro* plant cultures in the pharmaceutical, cosmetic and food industries. *In vitro* cultures can be a good alternative to field cultivation. Plant biotechnology allows for the control and optimization of the conditions for *in vitro* culturing to increase active compound accumulation. Among other things, it facilitates culture medium optimization, including the concentration of plant growth and development regulators, the use of elicitors (substances that mimic plant damage and facilitate secondary metabolites production, such as flavonoids), the selection of highly productive cell lines and genetic transformations. *In vitro* cultures can also be used in plant propagation (micropropagation process)[83].

Thus far, *C. limon in vitro* cultures have been the subject of research related to the development of micropropagation protocols. The focus has been on the selection of plant growth regulators (PGRs) that induce shoot and root production in *in vitro* cultures[84-87].

11 Bioavailavility of nutrients from *C. limon*.

The bioavailability of bioactive compounds depends primarily on chemical structure, matrix effects and interactions. Food processing can be used to stabilize and develop products with a longer shelf life; however, it can also be used to increase bioavailability. Bioactivity studies involve *in vivo* experiments in humans, although *in vitro* methods are useful for determining stability under gastrointestinal conditions. Most dynamic digestion models do not simulate the entire human gastrointestinal tract, but only the upper part. The more complex dynamic systems comprise two compartments, simulating the stomach and the small intestine. Other models include static *in vitro* reproduction of the oral cavity and saliva coupled to a computer-controlled dynamic gastrointestinal model with controlled conditions (temperature, enzyme secretion, pH, and peristaltic movements)[88].

Different formulation approaches, including absorption enhancers, structural transformation (eg, prodrugs, glycosylation), or pharmaceutical technologies (eg, carrier complexes, nanotechnology, cocrystals), have been developed to date to circumvent the problem of the low bioavailability of *C. limon* juice active flavonoids by improving their solubility and increasing their dissolution rate, as well as increasing their mucosal permeation, preventing their degradation or metabolism in the gastrointestinal tract and their direct delivery to the physiological tract. By using these strategies the pharmacokinetic behaviour of various flavonoids has been greatly improved as expected, which is beneficial to improve their biological activity and subsequent clinical application[89].

Another study related to Vitamin C bioavailability showed that the encapsulation of Vitamin C in new types of liposomes causes an increase in the Vitamin C bioavailability at physiological level, without compromising its potency at cellular level. The clinical study was conducted by comparison with free Vitamin C oral intake[90].

12 Conclusions

This review shows that *C. limon* and especially its fruit, lemon, is a very versatile food for the prevention and treatment of various pathological and age-related conditions. The *C. limon* fruit is a raw material that can be used in various ways: extract, juice and essential oil. The rich chemical composition of this species results in a wide range in its biological activity and its recommended use in phytopharmacology. Studies have focused on the essential oil and its main active compound: D-limonene. *C. limon* fruit extracts are rich in flavonoids such as naringenin, hesperetin and hesperidin.

Current pharmacological studies have confirmed *C. limon* fruit's healthy biological activities, especially its anti-cancer and antioxidant properties. *C. limon*'s applications in cosmetology and food production are also increasing.

The great potential of the *C. limon* fruit regarding efficacy and biological functionality provided by this review's bibliography shows that there is still a long way to go in terms of scientific knowledge about this versatile fruit. Among the wide variety of nutrients present in lemon, studies have focused on the most abundant nutrients such as Vitamin C or D-limonene. However, we find innumerable compounds that are potential nutrients that could demonstrate functionality and biological activity at higher concentrations than those found in lemon juice, such as hesperidin.

Many of the compounds present in lemon juice could present intestine absorption difficulties and, due to the matrix and the environment in which they are found, show a physiological effect. Physical instability, temperature, enzymatic environment or pH are factors to consider. In this regard, future studies, both *in vitro* and *in vivo*, should contemplate the following aspects to overcome the obstacles and, consequently, optimize these compounds bioaccessibility outside the dietary matrix of *C. limon*. Firstly, more efforts should be focused on modifying oral delivery systems by exploring convenient and safe techniques and procedures and by using natural biocompatible materials or carriers such as liposomes.

Secondly, many related studies are still at the laboratory stage, leading at best to *in vivo* studies in highly controlled groups of experimental animals. In these cases, it would be highly desirable to translate the laboratory's achievements into products and subsequently promote safety and efficacy clinical trials in groups of human individuals. This can be achieved by improving the payload of nutrients in oral intake matrices, the use of inexpensive excipients, and systematic investigation of *in vivo* stability, biodistribution, and disease treatment efficacy. We are convinced that, with rational design and continuous and innovative studies, a bright future can be foreseen for the active nutrients of *C. limon* and their potential in biological, pharmacological and food activities.

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