

Review

Essential Oils as a Feed Additives: Pharmacokinetics and Potential Toxicity in Monogastric Animals

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Simple Summary: Essential oils are regarded as possible substitutions of antibiotics. Some of them show strong antibacterial effects, and other positive effects in the nutrition of monogastric animals. The article aims to summarise the final state of the art concerning their pharmacokinetics in the organism. Last but not least, great attention is paid to their potential toxic effects.

Abstract: Essential oils (EOs) are now a hot topic in finding modern substitutes for antibiotics. Many studies have shown positive results and confirmed their high antibacterial activity both in vitro and in vivo. Deservedly, there is an attempt to use EOs as a substitute for antibiotics, which are currently limited by legislation in animal breeding. Given the potential of EOs, studies on their fate in the body need to be summarized. The content of EO's active substances varies depending on growing conditions and consequently on processing and storage. Their content also changes dynamically during the passage through the gastrointestinal tract and their effective concentration can be noticeably diluted at their place of action (small intestine and colon). Based on the solubility of the individual EO's active substances, they are eliminated from the body at different rates. Despite a strong antimicrobial effect, some oils can be toxic to the body and cause damage to the liver, kidneys, or gastrointestinal tissues. Reproductive toxicity has been reported for *Origanum vulgare* and *Mentha arvensis*. Several publications also address the effect on the genome. It has been observed that EOs can show both genoprotective effects (*Syzygium aromaticum*) and genotoxicity, as is the case of *Cinnamomum camphor*. This review shows that although oils are mainly studied as promising antimicrobials, it is also important to assess animal safety.

Keywords: phytogenics; natural growth promoters; antibiotic alternatives; genome

1. Introduction

Essential oils (EOs) are natural extracts, whose origin is folk medicine. In general, their use is eco-friendly, non-toxic and consistent with nature. Today's scientific research on EO efficiency is based on different traditional healing systems all over the world. Many preclinical studies have documented antimicrobial, antioxidant, anti-inflammatory, and anticancer activities. Except for the known toxic ingredients, EOs are considered generally regarded as safe (GRAS) in mammals [1,2]. Although many research activities are focused on the toxicity of EOs against insects [3] or aquatic organisms. EOs are unique in that each type differs in the composition of active substances and their concentrations. Currently, approximately 3000 substances in EOs have been identified. The chemical composition of EOs depends on the ambient conditions of plant growth and genetic diversity, which makes it difficult for exploration and commercial exploitation [4,5]. Their representation, combination and quantity

results in their properties and behaviour in the organism. Thus, breeding programs for pharmaceutical purposes seem to be a hot topic of research [6–8].

Recently, EOs has become an alternative to antibiotics in animal feed. Partly to prevent the antibiotic resistance of microorganisms, but also through the legislative restriction of the frequent use of zinc medication doses [9]. In several in vitro and in vivo studies, it could be found that extracts from rosemary, oregano, dill, cinnamon, eucalyptus, garlic, clove, or thyme were able to modulate ruminal methane emission to various extents, primarily by acting on methanogenic microflora [10]. The mechanisms of action have been summarized in many review articles [11–13]. Briefly, these publications describe that EOs components can disrupt the bacterial membrane, damage their metabolic processes or produce reactive oxygen species (ROS) and prevent the synthesis of bacterial toxins [14]. Conversely, some EOs have shown positive effects on microbes [15–17]. Moreover, the efficacy of EOs on poultry and swine production have been identified and reviewed [18–20]. The cellular protective effects of EOs against drugs or xenobiotics are also described in the literature [21–24].

The results of some studies demonstrated the efficacy of EOs as feed additives in animal breeding [25]. However, we have found several articles that address their potential toxicity. To provide a comprehensive overview and assess the safety of animals, we bring insights into the issues of their production and pharmacokinetics in mammal organisms.

2. Influence of EOs Production on Their Chemical Composition

Due to the EOs complexity, more recent attention has been focused on the choice of extraction procedure that could affect their yield and character. Currently, conventional methods (hydrodistillation, steam distillation, hydro diffusion, and solvent extraction) are alternated by green and sustainable extraction procedures. Benefits include shorter extraction times, lower energy consumption, low solvent usage, and less carbon dioxide emissions [26,27]. Gentle extraction approaches, such as CO₂ extraction, retain the antioxidant activity of active substances [28]. Nevertheless, successful extraction does not depend on the time of extraction but from an individual approach to the plant material [29]. It has been suggested that levels of the choice of extraction agent also plays a significant role [30]. Comparative studies of the extraction of various oils confirmed the high variability in the composition of the extracted substances that results in their varied effectiveness. The differences in extraction yield are mainly influenced by physicochemical parameters (temperature, time, pH, extraction dynamics), by the technique used or by the inclusion of other steps, such as ohmic heating, the assistance of ultrasound, or ionic liquids [31–39].

Factors found to be influencing EOs composition during storage have been explored in several studies. Notably, the exposure to EOs to atmospheric oxygen and UV radiation is one of the major causes of chemical change leading to loss of their efficiency [40–43]. It has been demonstrated that some phenolic components of EOs are oxidized by contact with reactive oxygen species (ROS) producing very reactive phenoxy radicals. These types of radical reactions are enhanced by the presence of transition metal ions, such as Fe²⁺, Cu²⁺, Zn²⁺, Mg²⁺, or Mn²⁺ [44–46]. In this regard, some studies have shown higher oxidative stability of ethanolic EOs. If storage temperature does not exceed 10 °C, then EOs will maintain their stability for up to eight weeks [47,48]. A growing body of literature has investigated the protection of EOs against oxidation. In this context, non-ionic surfactants, preservatives or stabilizers can be applied [49,50]. The effect has also been demonstrated in the use of gamma radiation with a dose of 20 to 30 kGy. Although results have confirmed an increase in antioxidant activity, there were reduced antibacterial effects due to changing certain active substances caused by gamma radiation [51]. Instead of chemical-physical protection against oxidation, the encapsulation seems to be one of the most perspective methods for preventing the stability changes [52]. Noori et al. used randomly methylated beta-cyclodextrin for encapsulation of *Zingiber officinale* EOs. Comparative tests have confirmed increased antibacterial activity against *E. coli* and *S. aureus*, and antioxidant activity [53].

3. EOs Pharmacokinetics in the Organism

An understanding of the pharmacokinetics of EOs in the organism is critical for consideration its effectivity and toxicity. The main obstacle to use EOs as an animal antibiotic is that the active substances enter the intestines at a concentration that is less than inhibitory. Commercial preparations often offer complex mixtures with bonded EOs or the oils are mixed into feed rations. During the digestion process, the individual active substances of EOs are degraded and metabolized. Generally, the kinetics of this process is based on the EOs composition.

EOs are complex mixtures of organic compounds including volatile compounds (such as monoterpene and sesquiterpene hydrocarbons, and their oxygenated derivatives) and of non-volatile residues (such as hydrocarbons, fatty acids, sterols, carotenoids, waxes and flavonoids) [54]. The solubility of the individual active substances has the greatest effect on absorption in the organism. In the gastrointestinal tract, the EOs compounds tend to interact with digested food. As a result, active substances could escape to solubilization and adsorption in the stomach. Moreover, the kinetics rate depends on the activity of digestive enzymes to release the EOs components from the fatty acid bonds [55].

Terpenoids and steroids (carotenoids, phylloxanthins, triterpenoids, and monoterpenes) show lipophilic character. Lipophilic molecules of EOs tend to form micelles, and they are digested in the small intestine together with other lipids. Moreover, their lipophilic character enables them to have easy penetration via the epithelial cell membrane. Thus, the molecule forms are delivered to the small intestine where are released and hydrolysed together with lipids [56,57].

Hydrophilic EOs components (polyphenols, flavones, flavanols, lignans, aromatic acids) are generally bonded to saccharides. These glycosides are metabolised in the small intestine, and their ligands are accessible to enterocytes. Non-absorbed aglycones metabolic pathway passes through the liver, where it is absorbed, and subjected to enzymatic degradation. Besides digestive processes, anthocyanins, isoflavones, tannins metabolism depends on their chemical nature (such as glycosylation) and intestinal microbiota [58–60]. Phenolic compounds are known to be unstable in GIT, and they are an easy subject to interaction with other food constituents, or they are hydrolysed during the small intestine passage. [61]. Free hydrophilic molecules are transported into enterocytes via passive diffusion or active transport in the duodenum.

A major proportion of EOs compounds are eliminated by renal excretion, as evidenced by an increase in urine analytes [62]. Non-absorbed and non-metabolized polyphenols leave the body in the faeces [60]. Some studies have reported that the highest concentration of active compounds from EOs is two hours after administration, and after five hours, the substances have been already effectively eliminated from the bloodstream. For example, the half-life of carvacrol, thymol, eugenol, and trans-cinnamaldehyde ranged between 1.84 and 2.05 h, whereas trans-cinnamaldehyde showed the fastest disappearance [63]. Moreover, it was found that the co-existing compounds in *Rhizoma Curcumae* extract could change the pharmacokinetic behaviours [64]. Mason et al. found that residues of the main of oregano and thyme EOs were present up to 13 days in dairy cattle [65,66]. It has been noticed that early absorption could lead to decreasing of antimicrobial effects in the gastrointestinal tract [66]. In this regard, more attention should be paid to the protection of active substances from the undesirable metabolic transformation.

4. EOs Toxicity

More recently, literature has emerged that offers contradictory findings of the toxicity of EOs in vitro and in vivo. The discussed health risk includes effects such as respiratory disorders, skin sanitiser, carcinogens, reproductive toxicity, or organ toxicity. In this context, the risk assessment should be taken into account; risk identification, dose-response evaluation, time of exposure, and mechanism of toxicity. The first phase of testing includes in vitro cytotoxicity tests of potentially harmed tissues. Subsequently, the tested substances could be applied in an organism.

EOs have always been used in traditional healing, making many people consider them safe. Although some toxic essences have been deliberately abused for their effects. For example, pennyroyal oil induces abortions and hallucinations, lemon oil is considered as a psychedelic [67,68]. EOs potential to substitute antibiotics are recognized as safe with a long tradition in herbal medicine. In many cases, highly aromatic oils were dosed at low concentrations, which do not cause any damage due to their difficulty to eat. However, poisoning by EOs has been recorded [69]. On the one side, their encapsulation or other kinds of masking their aroma could be risky. On the other side, some studies use the ability of encapsulated EOs to target release, transdermal transfer, enhance permeability effects, or for tissue targeting [70].

Toxicity assessment is complicated by the high variability of the active substance content, which can be up to hundreds in one type of oil [71]. Only a few articles found proposed mechanisms of toxicity. In general, the mechanisms of the cytotoxic effect of EOs are the production of ROS, blockade of sodium channels (e.g., for thymol 150 μ M IC50, skeletal muscle cells), cell cycle disruption, mitochondrial damage, DNA aberration, and initialization of NF- κ B cascade leading to apoptosis induction [72]. Studies conducted on spermatozoa confirmed that the most sensitive to the dose of EOs is sperm motility, and membrane integrity was minimally affected with a dose of *Melaleuca alternifolia* EOs at a concentration of 0.6 mg/mL [73]. Other oils, such as (*R. officinalis* and *T. capitata*), have also shown similar effects [74,75]. Another toxicological investigation has shown an interesting effect that EOs components in *Salvia officinalis* (α -pinene, camphene, limonene, 1,8-cineole, camphor, borneol, bornyl acetate, α -humulene, viridiflorol, humulene epoxide II, and manool) may exhibit dualistic pharmacological properties. The capability of both neurotransmitter excitatory and inhibition mechanisms in the control of anxiety [76]. Atsumi et al. has shown that the molecular structure plays a role in the toxicity of the active ingredients. Eugenol, the major component of clove EOs, shows much higher cytotoxic effects in its isoform [77]. Moreover, the toxic effects of EOs could be also given by minor ingredients, rather than the most abundant compounds [78]. The comprehensive overview of the studies is given in Table 1.

Table 1. Cytotoxic effects of EOs (essential oils).

EOs	Main Substances	Cell Line	Dose	Time	Ref.
<i>Lavandula angustifolia</i> <i>Helichrysum italicum</i>	linalool 28.9%; linalyl acetate 32.9% α -Pinene; camphane; β -Pinene; myrcene; p-cymene; borneol; thymol	human lymphocytes	0.3 L/mL	24 h	[79]
<i>Alpinia brevilabris</i> <i>Alpinia cumingii</i> <i>Alpinia elegans</i>	α -Pinene 10.1%; β -Pinene 35.3%; α -Pinene; β -Pinene; ρ -Cymene; α -Terpinene; α -Pinene; α -Cubebene α -Pinene; β -Pinene; ρ -Cymene; α -Terpinene; α -Pinene; α -Cubebene; 1,8-Cineol	human lung fibroblasts	IC50 90 μ g/mL IC50 70 μ g/mL IC50 30 μ g/mL	24 h	[80]
<i>Callicarpa micrantha</i> <i>Cinnamomum mercadoi</i> <i>Piper quinqueangulatum</i>	β -pinene; caryophyllene epoxide; aristolochene; borneol; linalool Cinnamaldehyde; Camphene; Linalool; α -phellendrene pinene; khusimene; cadinene		IC50 85 μ g/mL IC50 215 μ g/mL IC50 40 μ g/mL		
<i>Alpinia oxymitra</i> <i>Boesenbergia rotunda</i>	Epicatechin; Galloepicatechin 2',4'-dihydroxy-6-methoxychalcone; 5-hydroxy-7-methoxyflavanone; 5,7-dihydroxyflavanone	human lung fibroblasts	IC50 10 μ g/mL IC50 20 μ g/mL	24 h	[81]
<i>Cinnamomum cambodianum</i> <i>Citrus lucida</i> <i>Limnophila aromatica</i> <i>Rhodamnia dumetorum</i> <i>Sindora siamensis</i> <i>Cinnamodendron dinisii</i>	Cinnamaldehyde; Camphene; Linalool; α -phellendrene d-limonene z-ocimene 39.2%; terpinolene 17.2%; camphor 12.9% α -, β -, and γ -eudesmol; α - and β -pinene α -copaene 41.3%; β -cubebene 15.4%; β -cadinene 7.2% 1,8-cineole and sabinene.		IC50 180 μ g/mL IC50 15 μ g/mL IC50 2 μ g/mL IC50 6 μ g/mL IC50 30 μ g/mL		
<i>Gallsia integrifolia</i>	dimethyl trisulfide 15.49%; 2,8-dithianonane; 52.63%; lenthionine; 14.69%	Chinese hamster ovary cell lines	IC50 7 μ g/mL	72 h	[82]
<i>Schinus molle</i> L.	α -Phellandrene 45.7%; β -phellandrene 13.6%; Hmonene 13.4%; α -phellandrene 22.1%, β -phellandrene 10.4%; limonene 9.6%; α -cadinol 5.6%	human lymphocytes human macrophage	LD50 30.07 μ g/mL LD50 42.07 μ g/mL	72 h	[83]

Table 1. Cont.

EOs	Main Substances	Cell Line	Dose	Time	Ref.
<i>Citrus bergamia</i> <i>Litsea cubeba</i>	Limonene 37.2%; Linalyl acetate 30.1%; Linalool 8.8% citral; geraniol; neral.	mouse fibroblast cells	EC50 0.0023% v/v EC50 0.011% v/v	4 h	[84]
<i>Citrus X sinensis</i> <i>Cymbopogon citratus</i>	A-pinene; Citronellal; Geraniol; Limonene; Linalool; Myrcene; Neral citral A; citral B; neral		EC50 0.009% v/v EC50 0.013% v/v		
<i>Satureja sahendica</i> <i>Bornum</i>	Thymol 40%; gamma -terpinene 28%; and rho-cymene 22%	human cancer cell	IC50 15.6 µg/mL	24 h	[85]
<i>Rosmarinus officinalis</i>	1,8-cineole; a-pinene; camphor	dermal cell	IC50 5 mL/kg	72 h	[86]
<i>Piper aduncum</i>	pinene; khusimene; cadinene	erythrocytes	observed harmful effects 200 µg/mL	24 h	[87]
<i>Achillea millefolium</i> L.	thymol 26.47%	macrophages	IC50 22.11 mg/mL	24 h	[88]
<i>Thymus munbyanus</i>	thymol 52.0%; gamma-terpinene 11.0%; rho-cymene 8.5%; carvacrol 5.2%	human spermatozoa	500 µg/mL no observed toxicity	30 min	[89]
<i>Satureja khuzistanica</i>	Carvacrol 92.87%; limonen 1.2%	cancer cell lines	IC50 125 µg/mL	24 h	[90]

IC50 = half maximal inhibitory concentration; LD50 = median lethal dose; EC50 = half maximal effective concentration.

Although extensive research has been carried out on the antibiotic potential of EOs, there are a few studies elucidated their interaction with nucleic acids. The available studies show that there are two phenomena. At first, genoprotective effect against xenobiotics when EOs have been shown to prevent DNA mutations and aberrations [91–93]. Secondly, genotoxicity—mostly for those who are known for their toxicity such as *Syzygium aromaticum*, *Artemisia absinthium* or *Salvia divinorum*. Model eukaryotic organism *S. cerevisiae* have been analysed on genotoxic effects of *Origanum compactum*, *Artemisia herba alba* and *Cinnamomum camphora* EOs. The primary outcomes of the study suggested that EOs alone caused slight mutations in the cytoplasm, but not in the nucleus. In combination with mutagenic agents (UVB and 8-methoxypsoralen) plus UVA radiation and methylmethane sulfonate, cytoplasmic mutations and mitochondria damage were strictly increased, but nuclear DNA showed no mutagenic changes in combination with genotoxic treatment and EOs [94].

In Vivo Toxicity

In vivo assays are predominantly conducted to evaluate EOs potential to replace antibiotics both in animals and humans. An overview of publications that monitored the effects of EOs in vivo is given in Table 2. EOs are most commonly associated with hepatotoxicity, nephrotoxicity, changes in the blood vessels, and oxidative stress that occur as a result of acute intoxication. Some EOs have also been associated with reproductive toxicity. It was found that *Origanum vulgare* EOs at a concentration of 27% v/v affected the size of the genitals. The authors suggested a connection with the possible formation of metabolic syndrome and a decrease in testosterone levels. The highest dose also reduced sperm concentration and induced changes in Leydig cells. Thus, male rat fertility decreased as in a dose-dependent manner of the EOs [95]. These results were positively correlated with Shama A. J., who confirmed the contraceptive effects of *Mentha arvensis* at a dose of 10 mg/day/mice treated for 20–60 days. It is also known that some other EOs or their components affect the reproductive system. A new study demonstrated the prenatal toxicity of Verbena EOs. Embryo-fetotoxicity retardation was observed as evidenced by the decrease in foetal weight, head cranium, tail length, and higher incidence in the pre- and post-implantation loss. Some foetal skeleton abnormalities such as incomplete ossification of the skull, sternebrae, and metatarsal bones were observed in foetuses of the 2000 and 3000 mg/kg groups. Flavonoids such as apigenin and luteolin have been identified as major toxicants for the reported prenatal developmental toxicity [96].

In addition to actual toxicity, sub-chronic doses have been monitored. During the 90-day administration of carvacrol/thymol (10:1) at doses of 5, 100 and 200 mg/kg b.w./day, no animals died or showed deviations from the control group. The monitored parameters were overall health state, weight, feed consumption, blood count and histopathological findings. Only glucose levels were decreased, and females had enlarged ovaries in the treated group. The similar results were obtained

for *Pinus eldarica* EOs in the dose 125 and 250 mg/kg for 28 days and *Satureja khuzestanica* in the dose for 14 days 0.2–0.6 mL/kg [97,98]. These doses are also found to be tolerable in palatability studies, and also below the LD₅₀, which is approximately 2000 mg/kg.

Compared to antibiotics, EOs are tolerable at higher doses. In general, LD₅₀ for antibiotics ranges from units up to hundreds of mg/kg for acute administration. The sub-acute toxicity of most antibiotics brings a number of side effects affecting important internal organs. In addition, the discharging of the intestinal microbiota and the resistance of pathogenic microorganisms is still an unsurpassed negative side. Despite the enormous potential of EOs to serve as antibiotic admixtures or feeds, the Guidelines of the Scientific Committee on Food for Safety Assessment (EFSA, 2016) recommend genotoxicity and subchronic studies at the core of tests.

Table 2. Overview of the effects of EOs tested in vivo.

EOs	Main Substances	Organism	Dose	Effects	Ref.
<i>Syzygium aromaticum</i>	ugenol (64.74%), caryophyllene (14.36%), 3-Allyl-6-methoxyphenyl acetate (13.28%), 1,4,7, Cycloundecatriene, 1,5,9,9-tetramethy (2.55%).	rats	intraperitoneal injection, 0.125 mg/kg	higher levels of AST, ALT, ALP, decrease of AST hepatotoxicity	[21]
<i>Commiphora myrrha</i>	a-pinene, cadinene, limonene, cuminaldehyde, eugenol, m-cresol, heerabolene, acetic acid, formic acid	mice	injection 80 µL	pathological changes on liver and kidney, weight loss	[99]
<i>Calendula officinalis</i>	triterpenoid esters, carotenoids flavoxanthin, auroxanthin, lutein, zeaxanthin, flavonol glycosides, triterpene oligoglycosides, oleanane-type triterpene glycosides, saponins, sesquiterpene glucoside	rats	20 mL/kg body weight	higher levels of AST, ALT, ALP	[100]
<i>Mentha mozaffarianii</i>	α-Pinene 0.6%; Camphene 0.2%; Sabinene 0.5%; β-Pinene 1.0%; Myrcene 0.3%; Ocymene 0.6%; Limonene 0.4%; 1,8-Cineol 11.7%; Linalool 11.1%; Menthone 1.9%; δ-Terpineol 0.3; Borneol 1.0%; 4-Terpineol 0.2%; α-Terpineol 3.4%; Pulegone 0.3%; Piperitone 51.0% Thymol 1.0%; Piperitenone 8.6%; Piperitenone oxide 2.3%; Trans-Jasmone 1.9%; β-Caryophyllene 0.8%	rats	2000 mg/kg diet	higher level of glucose, cholesterol, ALT, AST, ALP, and TSH; tissue damage of liver, kidney, stomach	[101]
<i>Trachyspermum ammi</i>	Thymol 58.9%; p-cymene 24.02%; γ-terpinene 13.77 %; β-pinene 1.90%	mice	7% acute dermal irritation	defined erythema	[102]
<i>Boenninghausenia albiflora</i>	propyl ether 22%; linalool 22%; cinnamaldehyde 15%; cinnamyl alcohol 5%	rats	400 mg/kg diet	changes in the clinical picture (RBC, MCV, triglycerides, HDL, LDL, urea, and sodium)	[103]
<i>Cuminum cyminum L.</i>	Cuminaldehyde; cymene; terpenoids	rats	1000 mg/kg diet	increase of serum levels of ALT	[104]
<i>Satureja khuzestanica</i>	Carvacrol 11%; Thymol 28.2%; γ-terpinene 16%; p-cymene 19.6%; β-pinene 4.5%; Sabinene 4.4%	mice	injection 1.79 mL/ kg body weight	death	[98]
<i>Artemisia vulgaris L.</i>	Camphor; 1,8-cineole	rats	10.3–23.1 mg/kg body weight	anaemia	[105]
<i>Aquilaria crassna</i>	sabinene; linalyl acetate; anisaldehyde; perillaldehyde; 3-carvomenthenol; 3-carvomenthenone; bornyl acetate; p-mentha-1,3-dien-7-ol; cuminic acid; p-mentha-1,3-dien-7-av	mice	2000 mg/kg/day orally	weight loss	[106]
<i>Salvia officinalis</i>	Camphor 25%; 1,8-cineole 7.5%; α-tujone 22.2%	rats	30 mg/kg body weight	induced hepatotoxicity, lipid peroxidation	[107]

Table 2. Cont.

EOs	Main Substances	Organism	Dose	Effects	Ref.
<i>Curcuma longa</i>	cinnamic acid; 5 malonyl-CoA; p-coumaric acid	rats	5000 mg/kg body weight		[108]
<i>Piper vicosanum</i>	monoterpenoids 56.0–62.6%; limonene 40.0–45.5%; 1,8-cineole 10.4–15.0%	rats	2 g/kg body weight		[109]
<i>Lavandula angustifolia</i>	Linalool, Camphor and 1,8- cineole	mice, rabbits	2000 mg/kg diet		[110]
<i>Cinnamomum zeylanicum</i>	Cinnamaldehyde, Camphene, Linalool and α -phellendrene	mice	1.52 mL/kg body weight	No changes in the monitored parameters	[111]
<i>Origanum vulgare</i>	Carvacrol 80%; Thymol 64%; γ -terpinene 52%; ρ -cymene 52%	rats	200 mg/ kg body weight		[112]
<i>Satureja khuzestanica</i>	Carvacrol 11%; Thymol 28.2%; γ -terpinene 16%; ρ -cymene 19.6%; β -pinene 4.5%; Sabinene 4.4%	mice	0.2, 0.4 and 0.8 mL/ kg diet		[98]
<i>Piper glabratum</i>	pinene 12.0%; khusimene 12.1%; cadinene 13.2%	mice	5000 mg/kg/body weight		[113]
<i>Lavandula stoechas</i>	Linalool, Camphor and 1,8-cineole	rats	200 mg / kg body weight		[114]
<i>Ocimum sanctum L.</i>	Oleanolic acid, Ursolic acid, Rosmarinic acid, Eugenol, Carvacrol, β -caryophyllene and β -elemene.	mice	LD50 4571.43 μ L/kg	death	[115]
<i>Mentha mozaffarianii</i>	Linalool 51.8%; Epoxyocimene 19.3%; Sesquiphellandrene 9.4%; Cadinene 4.0%	rats	LD50 greater than 2000 mg/kg	increases blood glucose, cholesterol, ALT, AST, ALP, and TSH	[101]
<i>Lavandula stoechas</i>	1,8-cineole; lavandulol; necrodane	rats	200 mg/kg	No changes in the monitored parameters	[114]
<i>Origanum vulgare</i>	Carvacrol 80%; Thymol 64%; γ -terpinene 52%; ρ -cymene 52%	rats	3% diet	No changes in the monitored parameters (spermatozoa)	[95]
<i>Origanum vulgare</i>	Carvacrol 80%; Thymol 64%; γ -terpinene 52%; ρ -cymene 52%	rats	200 mg/kg b.w.	Data revealed no mortality and no treatment-related adverse effects of the EOs in food/water consumption, body weight, haematology, biochemistry, necropsy, organ weight and histopathology.	[112]
<i>Ocimum gratissimum</i>	Oleanolic acid; Ursolic acid; Rosmarinic acid; Eugenol; Carvacrol; β -caryophyllene; β -elemene	rats	1500 mg/kg body weight	No changes in the monitored parameters (functional damages to stomach and liver)	[116]
<i>Thymbra capitata (L.)</i>	1,8-cineole 19.60%; Camphor 17%; α -pinene 15.12%; Borneol 8.17%; Verbenone 9.55%	boars	0.6 mg/mL	No changes in the monitored parameters (spermatozoa)	[74]
<i>Eucalyptus staigeriana</i>	Cineole 46.8%; α -pinene 28.9%; d-limonene 4.9%	rats	LD50 3.495.9 mg/mL	death	[117]
<i>Eucalyptus Eucalyptus</i>	Cineole 6.2%; α -pinene 8.3%; ρ -cymene 28.6%; Cryptone 17.8%; Cuminaldehyde 6.5%	rats	LD50 2.334 mg/kg b.w.	death	[118]
<i>Eugenia caryophyllus</i>	eugenol; isoeugenol; eugenone; β -caryophyllene	rats	LD50 3.597 mg/kg b.w.	death	
<i>Pinus eldarica</i>	Thymol 78.8%; karvarol 6,2%	rats	LD50 higher than 22.5 mL/kg b.w.	No changes in the monitored parameters	[119]

Table 2. Cont.

EOs	Main Substances	Organism	Dose	Effects	Ref.
<i>Verbena officinalis</i>	1-octen-3-ol 30.76%; Verbenone 20.49%	pregnant female rats	3000 mg/kg diet	asymmetrical distribution of implantation sites and embryos	[96]
<i>Verbena litoralis</i> Kunth	Epicatechin; Galloepicatechin; Cadinene	rats	400 mg/kg diet	only increase in AST	[120]
<i>Lantana camara</i>	bicyclogermacrene 19.4%; isocaryophyllene; 16.7%; valencene 12.9%; germacrene D 12.3%	guinea pigs	24 mg/kg b.w.	decrease in weekly body weights, haematology, liver and kidney marker enzymes (ALT, AST, ALP and creatinine)	[121]

AST = aspartate transaminase; ALT = alanine transaminase; ALP = alkaline phosphatase; TSH = thyroid stimulating hormone; RBC = red blood cell count; MCV = mean corpuscular volume; HDL = high-density lipoprotein; LDL = low-density lipoprotein.

5. Conclusions

As a result of the global increase in the demand for antibiotics, EOs are continually being tested for their antimicrobial effects. Several methods for their production have been invented as well as measures to eliminate the extraction of antinutritional or toxic substances contained in natural flavours. Development and research are also growing in the field, for their use in controlling pathogens, or their pharmacological use. The weakness of this research is so far insufficient information about their metabolism in the organism. Some hydrophilic oil components are already absorbed in the stomach, whereas lipophilic oils can pass to the intestine where their site of action is most predicted. The chemical nature of some active substances predisposes them to interactions with other food ingredients and thereby deteriorating their availability. Encapsulation and the use of nanotechnologies seem very promising in this direction. Speculation also leads to their toxicity. Because they are considered GRAS, there is no attempt to investigate their harmful effects on the body, which can cause adverse reactions to organisms. EOs are a considerable issue that needs to be explored in broader contexts and involving more disciplines.

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