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THE ESSENTIAL OIL OF TURPENTINE AND ITS MAJOR VOLATILE FRACTION (α - AND β -PINENES): A REVIEW

BEATRICE MERCIER¹, JOSIANE PROST¹, and MICHEL PROST²

¹ Université de Bourgogne, Dijon, France

Faculté des Sciences de la Vie

² Lara-Spiral SA, Couternon, France

Abstract

This paper provides a summary review of the major biological features concerning the essential oil of turpentine, its origin and use in traditional and modern medicine. More precisely, the safety of this volatile fraction to human health, and the medical, biological and environmental effects of the two major compounds of this fraction (α - and β -pinenes) have been discussed.

Key words:

Spirits of turpentine, α -pinene, β -pinene

ORIGIN OF TURPENTINE

The term “essential oil of turpentine” designates the terpenic oil, obtained by hydrodistillation of the gem pine. It is also named the “spirits of turpentine”, “pine tree terpenic”, “pine oleoresin”, “gum turpentine”, “terpenes oil” or “turpentine from Bordeaux”. Due to its pleasant fragrance, the terpenic oil is used in the pharmaceutical industry, perfume industry, food additives and other chemical industries (household cleaning products, paintings, varnishes, rubber, insecticides, etc.) [1].

TRADITIONAL MEDICINE AND TURPENTINE

The eminent doctors of antiquity, Hippocrates, Dioscoride or Galien, used the terpenic oil for its properties against lung diseases and biliary lithiasis. In France, Thilenius, Pitcairn, Récamier and Martinet recommended it against the blennorrhoea and cystitis. Chaumeton, Peschiez, Kennedy, Mérat prescribed it against the

neuralgias. It was also used in the treatment of rheumatism, sciatica, nephritis, drop, constipation and mercury salivation.

Those scientists also recognized that the terpenic oil may be a booster at an average dose and may have a paralyzing activity at high doses. In Germany, (Rowachol and Rowatinex), Slovenia (Uroterp) and Poland (Terpichol and Terpinex), the traditional drugs for renal and hepatic diseases (especially against cholesterol stones in the gall bladder and the bile duct) contain α - and β -pinenes [2]. Modern phytotherapy describes the following properties of the terpenic oil: antiparasitic, analgesic, revulsive, disinfectant (external use); balsamic, active on bronchial secretion and pulmonary and genito-urinary tract infections, haemostatic, dissolving gallstones, diuretic, antispasmodic, antirheumatic, deworming, being an antidote for poisonings caused by phosphorus [3] and improving the ciliary and secretory activity in patients who present chronic obstructive bronchitis (internal use) [4].

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Address reprint request to B. Mercier, UPRES EA 4183 “Lipides & Signalisation Cellulaire”, Faculté des Sciences de la Vie, Université de Bourgogne 6, Boulevard Gabriel, F-21000 Dijon (e-mail: beamercier@laposte.net).

At present, the Vidal drugs Compendium lists 14 different drugs containing turpentine as active molecules, and 4 drugs containing turpentine as an excipient.

OXIDIZED TURPENTINE

Perfumes are, next to nickel, the most common allergic substances in the world. This property is connected with the fact that the majority of oils are sensitive to oxidation [5]. However, in some oils, like in the Indian plant *Chaulmoogra* [6] as well as in turpentine, the ageing/oxidation contributes to the therapeutic effect of these compounds, although they have the highest peroxide index of all terpenes [7]. For example, old oxidized terpenes become water-soluble (instead of lipid-soluble) and are able to capture and deliver oxygen (the property known since Berthelot's studies [8]). Thus, they can enhance the saturation rate of HbO₂ [9] or PaO₂ [10], which was confirmed by Mercier et al. in their study on "Bol d'Air Jacquier®", a modern device using oxidized turpentine vapour to combat cellular hypoxia [11,12]. Oxidized turpentine is considered to be an anti-inflammatory agent [13] and its peroxidized form is thought to exhibit an antiradical activity [12,14–18]. It seems to be a general trend that the essential oils which contain monoterpene hydrocarbons, oxygenated monoterpenes and/or sesquiterpenes have a higher antioxidative potential [19].

At high concentrations or when combined with a secondary organic aerosol, these oxidized products may be pro-inflammatory [20] and cause weak or moderate irritation with time [21]. However, acute and chronic toxicities in experimental animals (rat, rabbit) are low, and they refer to higher doses than those envisaged for therapeutic purposes [11]. It is interesting to underline that when administered at low doses, they possess remarkable properties. Specifically, they are used as relaxants of the smooth muscles of the bronchi ("Ozothin®") [22] as well as disinfectants and bactericides [23]. They also decrease PpCO₂ in hypercapnic patients (in whom the treatment does not generate hyperventilation) and improve the redox system activity and tissue diffusion [23], particularly when this substance is administered as an aerosol [24] with a broncho-secretolytic

action [8]. They also reduce cough and help cure respiratory diseases, particularly chronic bronchitis or asthma [23].

Table 1. Summary of the properties of terpene oxides reported in scientific literature

Oxidized terpenes
Relaxing smooth muscles
Acting as cough preservatives
Relieving congestion
Acting as anti-inflammatory agents
Having disinfectant properties (bactericidal)
Increasing HbO ₂
Increasing O ₂ arterial partial pressure
Increasing O ₂ tissue diffusion
Increasing the redox system activity
Being water-soluble

ENVIRONMENTAL IMPACT OF THE VOLATILE TURPENTINE FRACTION

The most volatile components of turpentine are two terpenes: alpha (α) and beta (β) pinenes. They are the dominant odorous compounds emitted by trees, shrubs, flowers and grasses [25].

In the lower troposphere, and depending on the weather conditions at the top of the trees, these compounds can react with OH° radicals, ozone, NO₃ radical and O₂. Indeed, the electric field in the canopy atmosphere (at the uppermost level of the pine, fir and spruce forests) is sufficient to produce discharges, particularly in stormy weather, or more generally, in wet weather, through which ozone (O₃) and hydrogen peroxides (H₂O₂ [26]) are released. Ozone also forms during sunny weather, particularly in the summer and autumn [27]. Reactions in both these conditions result in the generation of aerosols in the ultrafine particle form [28] as well as of peroxides (hydrogen peroxides and organic peroxides), carbon monoxide (CO), acid rains (starting from organic acids, of NO₃ and SO₄²⁻), ozone, or oxidizing radicals, like the OH° radical.

Winterhalter et al. [29] showed that in the presence of volatile organic compounds, such as NO_x or OH° radicals,

α -pinenes undergo an ozonolysis and transform mainly to acid products (cis-pinic, cis-pinonic and hydroxy-pinonic acids). These acids can react with primary pollutants like cyclohexene, propanol or formaldehyde [30] and, in general, photocatalysts like anthracene or zinc oxide [31].

Beta-pinens can also generate organosulfates or nitroso-organosulfates [32]. Due to the presence of a double bond between two atoms of carbon, the volatile monoterpenes are very reactive. According to the authors cited above, they could be the origin of secondary pollutants. Following other researchers, they rather seem to be natural scavengers of hazardous substances such as ozone [33].

Apart from the roles specified above, the volatile parts of turpentine exhibit several other properties, like an allelopathic activity [25]. Traumatic resinosis (mechanical wounding, abiotic stress, insect attack, pathogen invasion, elicitor molecules derived from fungal or plant cell walls [34]) make the volatile fraction of turpentine acquire insecticidal, acaricidal, "pesticidal" and/or insect repellent properties, according to the type of predator. This fraction plays a role in attracting pollinators [35]. The radical scavenging properties may contribute to the defense potential of the plant against pests [36].

Volatile fraction of turpentine (α - and β -pinenes)

Volatile pinenes of turpentine enter the body through inhalation but also through the skin, with a good correlation between the level of contamination of particular body parts and the potential body exposure [37]. The ability of volatile pinenes to penetrate through the skin, the low irritancy potential and the inclusion in the list of the substances that are Generally Recognized as Safe (GRAS), make it possible to use them as a support to increase the absorption of various chemicals. They are used, for example, for enhanced neuroleptic drug absorption [38]. The mechanisms responsible for enhancing the percutaneous activity of terpenes have been explained elsewhere [39].

The turpentine respiratory sessions considerably increase the capacity of the organism to transform the xenobiotics at the hepatic level, by increasing the activity of the NADPH cytochrome C reductase and the 7-ethoxycoumarin de-ethylase [40].

Alpha-pinenes

The effects of α -pinenes vary depending on the composition of monoterpenes and sesquiterpenes. Scientific research is generally related to the whole compounds rather than the molecular level because under natural conditions, it is always a family of terpenes that the plant generates. In addition, the biological effect is often due to a synergy between the compounds [41]. This explains many contradictions in the reported results: for example, α -pinenes are the major components of the Amazonian plant *Cordia verbenacea* spp. (approximately 27%). This plant possesses a remarkable effectiveness against Gram-positive bacteria and yeasts, but not against Gram-negative species [42]. However, other studies report the antibacterial effect of these terpenes on both Gram-negative and Gram-positive bacteria as well as a strong antifungal activity [43].

The mechanism through which α - and β -pinenes are active against yeast or bacteria lies mainly in their capacity to induce toxic effects on the membrane structure and functions [44]. We know that the cytoplasmic membranes of bacteria and the mitochondrial membranes of yeast provide a barrier to the passage of small ions such as H^+ , K^+ , Na^+ and Ca^{2+} and allow the cells and organelles to control the entry and exit of different compounds. This role of the cell membranes as a permeability barrier is integral to many cellular functions, including the maintenance of the energy status of the cell, other membrane-coupled energy transducing processes, solute transport, regulation of metabolism, and control of turgor pressure [45]. Sik-kema et al. [46] showed that due to their lipophilic character, cyclic monoterpenes will preferentially partition from an aqueous phase into membrane structures. This results in membrane expansion, increased membrane fluidity and inhibition of a membrane-embedded enzyme. In yeast cells and isolated mitochondria, α -pinenes and β -pinenes destroy cellular integrity, inhibit respiration and ion transport processes and increase membrane permeability [44]. More recently, Helander et al. [47] have described the effects of different essential components on outer membrane permeability in Gram-negative bacteria.

Thus, terpenes, containing the first or the second largest part of α -pinenes, fight against pathogenic bacteria and

all kinds of fungi. They are able to eliminate the microorganisms or inhibit their growth as well as intervene on their metabolism (for example, by preventing methane oxidation in the bacterium *Methylobacter luteus* [48]). They form an essential oil which is particularly effective on Gram-positive bacteria, including *Clostridium perfringens*, *C. sporogenes*, *Staphylococcus aureus* [36] and *S. epidermidis* [49].

Alpha-pinenes constitute a large part of the active solutions used against the Gram-negative bacteria, particularly on the strains responsible for jaw infections, parodontitis or periodontitis (*Actinobacillus actinomycetemcomitans*, *Prevotella intermedia*, *Porphyromonas gingivalis*, *Fusobacterium nucleatum* [50], *Yersinia enterocolitica* [36], *Salmonella typhi* [36], *Proteus vulgaris* [36,43], and *Acetobacter* spp. [36]. On the other hand, the results obtained for α -pinenes used to destroy *Escherichia coli* vary undoubtedly, depending on the bacterial strain (there exist the pathogenic and non-pathogenic strains) or on a possible synergy with other monoterpenes. Pichette et al. [49] demonstrated the inefficacy of α -pinenes; Magwa et al. [36] and Cha et al. [50] showed otherwise.

Moreover, α -pinenes are used against mushrooms and yeasts (dermatophytes [44]), especially on *Candida albicans* [36] and other related species such as *Candida tropicalis*, *C. glabrata* [60], *Aspergillus* spp. [36], *Cryptococcus neoformans* [43], *Penicillium notatum* [36], etc.

Finally, α -pinenes also act as insecticides, especially on mosquitoes like *Culex pipiens* causing paludism, and Nile fever vector [34], or on dengue's vector *Aedes aegypti* [51]. They act on the eggs of *Pediculus humanis capitis*, and against the female cockroaches, even if their effectiveness is lower than that of β -pinenes [52]. From another point of view, α -pinenes appear to have an inhibitory effect against *Pityogenes bidentatus*, or bark beetle [53].

In addition to their high effectiveness in controlling vectors of various diseases and parasites of all kinds, α -pinenes also exhibit certain biological effects: pre-treatment with α -pinenes decreases the hexobarbital sleep time of female rats by inducing microsomal enzyme activity [54]. They are inhibitors in breast cancer, and *in vitro* present cytotoxic activity against human cancer cells [12,55], but

not on the healthy cells like the red blood cells [12,18] or whole organisms [12,25]. Mercier [12] demonstrated that the peroxidizing α - and β -pinenes decrease the antiradical capacity of abnormal Jurkat cells. For Diaz et al. [56], the α - and β -pinenes were cytotoxic on several cell lines (breast cancer and leukemic cell lines). Zhou et al. [57] reported that α -pinenes are especially involved in the inhibition of the human monocyte factor NF- κ . On the other hand, Lampronti et al. [58] did not note an antitumor effect of the α - or β -pinenes alone. They did not exclude a possible synergistic effect with other monoterpenes, or sesquiterpenes like caryophyllene. All these studies provide further insight into the potential use of terpenes or a mixture of terpenes as the inducers of apoptosis in cancer cells.

Terpenes are also antioxidants [12], but Grassmann et al. [59] postulated that this antioxidant activity is effective only in a lipophilic environment. These compounds also possess anti-inflammatory properties [60] and exert spasmolytic and myorelaxant activity on the smooth muscles of the intestine [61]. These effects may explain their traditional use in the German and Polish traditional drugs for colic, diarrhea, cough and asthma. This antispasmodic activity would be due to the inhibition of the calcium channels. They also exhibit antinociceptive and antistress activity in rats [62]. However, the activity of α -pinenes was observed only at low doses. At higher doses, their activity referred to a number of neurological mechanisms regulating the cardiac function, which were activated or not, depending on the level of pinenes in the organism. From these findings, there derives a hypothesis of a different and pinene dose-dependent neurological response mechanism of the cardiac function.

As reported by Umezu et al. [63], experiments on mice indicated that the antistress property was reserved for other monoterpenes. However, they could not exclude a synergistic effect between all the components of the essential oil of lavender which was tested.

We also observed a regeneration of the β cells of the islets of Langerhans in the pancreas, resulting in the decrease of glycaemia, in a study investigating *Nigella sativa* L., a plant

containing α -pinenes [63]. Mercier [12] showed in *in vivo* and *ex vivo* studies a decrease in the rate of glycosylated haemoglobin after inhalation of oxidised turpentine vapours. Some studies investigating the effect of various terpenes, including α -pinenes, documented that the level of induced CYP2B, as measured by immunoassay, increased several times. Furthermore, CYP2B activity increased when laboratory rats were given an oral dose of α -pinene [64]. There is no evidence for induction of CYP3A with α -pinene [65]. Several essential oils are used for their memory-enhancing effects in the European folk medicine. Among the components, α -pinenes were found to inhibit AChE in an uncompetitive and reversible manner when they acted synergistically. They were responsible for the inhibitory effect of the essential oil of the *Salvia* species. Thus, when in synergy with other compounds, they could be beneficial in the treatment of cognitive impairments, due to their multifarious activities related to Alzheimer's disease [65].

It has also been shown that α -pinenes do not exhibit an oestrogenic activity [60] or a behavioural effect [63].

Table 2. Summary of the properties of α -pinenes, alone or in synergy with other pinenes, reported in scientific literature

α -pinenes
Lipophilic
Bactericidal
Fungicidal
Insecticidal
Pesticidal
Anticarcinogenic (cytotoxic on cancer cells)
Diuretic
Antioxidant
Immunostimulant
Anti-inflammatory
Anti-convulsive
Sedative
Anti-stress
Hypoglycaemic
Capable of expelling xenobiotics
Anticholinesterase activity

Beta-pinenes

According to literature reports, β -pinenes generally accompany α -pinenes in low quantities in the volatile extracts, essential oleoresins and oils, *i.e.* all the pine extracts which were tested for their biological properties. Some specific studies show that β -pinenes, along with α -pinenes and other terpenes, are cytotoxic on cancer cells [55]. They represent a great part of essential oils with sedative properties [66]. When α - and β -pinenes are the major constituents of an essential oil, they warrant the anti-inflammatory and analgesic activity [67].

The β -pinenes also show antifungal properties [68], especially on *Candida* spp. [36]. When acting on yeast, they were found to inhibit mitochondrial respiration, the proton pump activity and K^+ transport, and to increase membrane fluidity [69]. They also exhibit pest-destroying properties against the protozoon *Plasmodium berghei* (malaria vector [70]), insecticidal properties against lice [71] and the mosquito *Aedes aegypti* [51] as well as an antiseptic effect on oral bacterial flora [50]. In general, they exert a considerable antibacterial effect, especially on a methicilline-resistant *S. aureus* and other Gram-positive and Gram-negative bacteria [43].

Without α -pinenes, but with other terpenes, β -pinenes present antiradical activity (DPPH system [72] and elimination of the superoxide anion [73]). They belong to the essential oils used against the osteoclast activity (they thus play a protective role against osteoporosis [74]).

Beta-pinenes, when administered alone, exhibit moderate antimicrobial activity [68]; they are sometimes ineffective on specific strains like *Pseudomonas* spp. [75]. As the major or important components of essential oils, they are particularly powerful on fungus, like *Trichoderma* spp. [76]. Takikawa et al. [77] showed that β -pinenes acted on a pathogenic strain of *Escherichia coli*, but this activity is less pronounced against the non-pathogenic strains. Besides, β -pinenes show an insecticidal activity against the third larval stage of the fly *Musca domestica* [78]. In a synergistic activity with other terpenes, they act against the fruit fly, *Bemisia argentifolii* [79]. In competition with α -pinenes, they seem more active as antifungal agents (on *Fusarium culmorum*, *F. solani* and

F. poae, fungal phytopathogens [80]) and as inhibitors of *Brassica campestris* germination (colza), in a dose-dependant manner [81]. Beta-pinenes showed a better effectiveness than did α -pinenes in fighting against cockroaches [82].

In addition, the compounds are active on the smooth muscles of the ileum part of rat intestine. They act by inhibiting the 5-HT₃ receptors of the serotonergic system of the murine intestinal cells [83].

In rats, β -pinenes exert an antinotoxic effect on the supra spinal parts, but not on the spinal cord itself [28].

Table 3. Summary of the properties of β -pinenes, alone or in synergy with other pinenes, reported in scientific literature

β -pinènes
Lipophilic
Bactericidal
Fungicidal
Insecticidal
Acting against osteoclasts
Anticarcinogenic (cytotoxic on cancer cells)
Pesticidal
Antioxidant
Sedative

Harmlessness of the turpentine vapours

Some turpentine varieties, especially those originating from the Scandinavian countries, Switzerland, Germany or Italy, generate various types of allergies. Monoterpenes are released in the form of gas during the sawing and processing of fresh wood. They pose a potential health hazard for workers at sawmills [84]. They cause irritation to the skin, eyes and mucous membrane. They may be associated with the development of contact dermatitis (allergic or non-allergic) [85].

Turpentine inhalation increased resistance of the upper airways and induced chronic irritation, but did not generate acute respiratory problems [86].

Foussereau [85] observed 12 cases of eczema following the use of the Swedish turpentine instead of the French

turpentine. Likewise, 14 other people were cured of eczema by using turpentine without δ -3-carene (cases observed between 1967 and 1969 in Strasbourg).

In fact, the greatest danger related to turpentine use seem to be the δ -3-carenes, a variety of terpenes. These chemical compounds were at the origin of dermatitis and respiratory problems as they induced broncho-constriction [87], and there is a dose-dependent relationship between the viability of alveolar macrophages and the concentration of δ -3-carenes. They appear to have provoked a stronger reaction than did α -pinenes [88].

For the α - and β -pinenes (the main volatiles monoterpenes), many authors have a moderate opinion regarding their irritant capacity. First of all, a significant quantity is needed for the product to produce adverse health effects: as reported by Menezes et al. [89], the toxic effect for the mice starts at 5 g/kg. Kasanen et al. [90] postulated that it is highly unlikely that monoterpenes alone can cause irritation under normal conditions (“all pinenes possess sensory irritation properties and also induced sedation and sign of anaesthesia but had no pulmonary irritation effects”). Fransman et al. [91] observed that the respiratory problems referring to laminated wood workers were associated with the presence of formaldehyde among all the agents that these people inhaled at work (dust, bacterial endotoxins, abietic acid, formaldehyde and terpenes). Dutkiewicz et al. [92] found out that dermatitis among Polish workers resembled that characteristic of exposure to oak and pine wood dusts, and concluded that the presence of this pathology was due to dust inhalation rather than the composition of these dusts.

Thus, to paraphrase Paracelse, “Sola dosis facit venenum”, which translates as “the dose makes the poison”. The rate of irritation from terpene exposure correlates with exposure level. Accordingly, monoterpenes become pro-oxidants at higher doses [93]. At a low dose, they are included in the composition of pharmaceuticals used for the kidney and liver disorders [2]. At high-level exposure, they are hepato- and nephrotoxic. They can also cause nervous system disorders (convulsions, disorders of balance [26]).

IN VIVO EVOLUTION OF α -PINENES

The *in vivo* oxygenated derivatives of terpenes are terpineols, which have been used for centuries in the traditional medicine and perfumery. Currently, there are more than 22 000 terpineols known for their biological properties such as antioxidative activity, influence on immune functions, and anticancer potential [94].

Scientific studies, especially those of the Scandinavian researchers, made it possible to identify the absorption pathways of these monoterpenes in the body. As reported by Falk et al. [95], approximately 60% of the inhaled α -pinenes are eliminated in blood and show a high affinity with fat tissue. They are also eliminated through the lungs (8%) and to a lesser degree (0.001%) through the kidneys [96]. Filipsson [97], in his report on a two-hour inhalation of turpentine solution at the rate of 450 mg/m³, demonstrated that α -pinenes are eliminated in exhaled air (3% to 5%) and that their half-life (clearance) in blood is approximately 32 hours. The remaining part is metabolized by hydration and hydroxylation (this degradation is universal, from bacteria to mammals). These reactions take place at the hepatic cells level, and especially at the P₄₅₀ cytochrome level [98]. The metabolites are excreted in urine [19,20,96,97].

Also known is the transformation of α -pinenes into limonene, myrtenol, oxidized α -pinene and pinocarveol (List of All UM-BBD Biotransformation Rules, Minnesota University, USA). The process is widespread in the entire world, starting from fungus to vegetal cells or bacteria. In mammals, the most common chemical evolution of α -pinenes is their hydroxylation to verbenol (C₁₀H₁₆O), and, also to myrtenol and myrtenic acid [98,99] (Fig. 1).

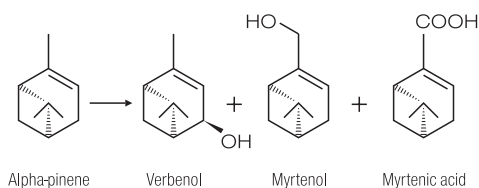


Fig. 1. Alpha-pinene evolution in mammals [98].

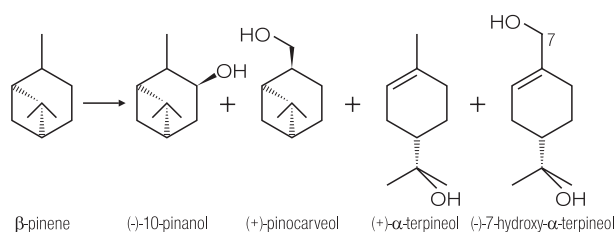


Fig. 2. Beta-pinene evolution in mammals [98].

The transformation from a “terpene” form to a “terpineol” form also means new biological properties. The new molecules exhibit the following biological activities: the *cis*-verbenol is an antioxidant [9], it prevents the resorption of osteoclasts, thus having a positive effect on osteoporosis (the α -pinenes do not possess this property [74]), and it is active against *Escherichia coli*, *Staphylococcus aureus*, and *Bacillus subtilis* [36].

IN VIVO EVOLUTION OF β -PINENES

Filipsson [97] reported that approximately 66% of β -pinenes are eliminated in blood (two-hour inhalation of turpentine solution at the rate of 450 mg/m³) and that their half-life in blood is approximately 25 hours (their rate of elimination is higher than that of α -pinenes). The evolution scheme of β -pinenes is displayed in Figure 2 [98]. The hydroxylated products also exhibit new biological properties. For example, the α -terpineol exerts an anti-inflammatory activity by reducing the rate of TNF α , interleukins IL-1 β , IL-8, IL-10 and prostaglandins E₂ (α -terpineol is the major component of the essential oil of the tea tree *Melaleuca alternifolia* [100]).

CONCLUSION

The essential oil of turpentine and its two major volatile compounds are natural products, which pose no hazard when used in small quantities. They have a number of properties that are beneficial to human health and wellbeing and may be used in the pharmaceutical and cosmetic industries. The major characteristics of these compounds are summarized in Figure 3.

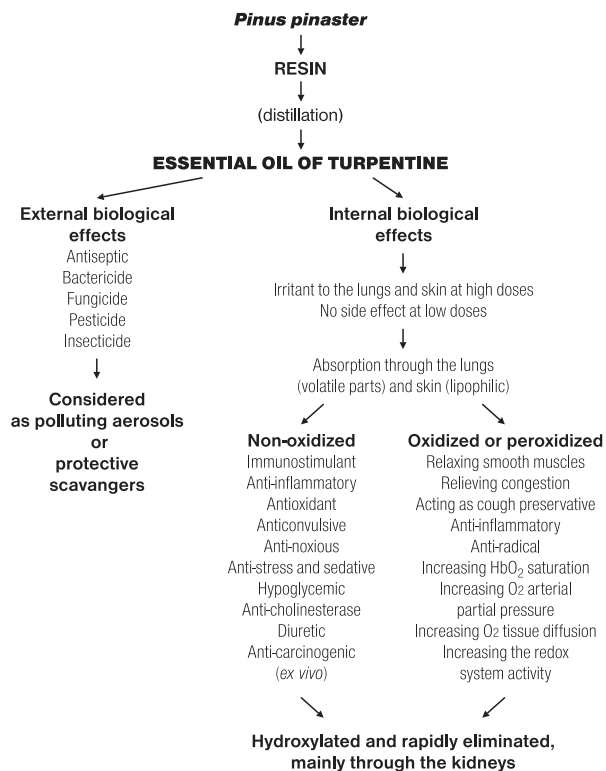


Fig. 3. Origin, impact and elimination of the essential oil of turpentine and its two main volatile components.

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