A Scoping Review on the Therapeutic Potential of Resin From the Species *Larix decidua* Mill. [Pinaceae] to Treat Ulcerating Wounds

João V. C. Batista¹,², Annekathrin Uecker³, Carla Holandino⁴, Fabio Boylan⁵, Jakob Maier¹, Jörg Huwyler² and Stephan Baumgartner¹,³,⁶*

¹Hiscia Institute, Society for Cancer Research, Arlesheim, Switzerland, ²Department of Pharmaceutical Sciences, Division of Pharmaceutical Technology, University of Basel, Basel, Switzerland, ³Institute of Integrative Medicine, University of Witten/Herdecke, Witten, Germany, ⁴Departamento de Fármacos e Medicamentos, Faculdade de Farmácia, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil, ⁵School of Pharmacy and Pharmaceutical Sciences, Trinity Biomedical Sciences Institute, Trinity College Dublin, Dublin, Ireland, ⁶Institute of Complementary and Integrative Medicine, University of Bern, Bern, Switzerland

Malignant ulcerating wounds or neoplastic lesions are a considerable burden for patients suffering from advanced cancer. These wounds have no effective treatment and are very difficult to manage. The present review summarizes evidence in support of a hypothesis put forward in anthroposophic medicine, which suggests a beneficial role of resin from the species *Larix decidua* Mill. [Pinaceae] for treating such wounds. A systematic search strategy was performed using the databases PubMed, EMBASE and SciFinder. The included publications described the chemical composition of this species, as well as in vitro, in vivo, and ex vivo experiments using plant extracts and isolated compounds. The results show that among the phytochemical classes, terpenoids were the major components of this species, especially in the resin. The summarized biological experiments revealed antimicrobial, antioxidant and anti-inflammatory effects, with promising potential for the extracts and isolated compounds. However, the molecular mechanisms and toxicological effects are as of yet not conclusively evaluated. From the data of our study, we can conclude that *L. decidua* might indeed have a promising potential for the treatment of malignant wounds, but definitive information that can prove its effectiveness is still lacking. We therefore suggest that future efforts should be dedicated to the evaluation of *L. decidua* resin’s therapeutic use considering its antiseptic action and proposed wound healing properties.

Keywords: Larch resin, *Larix decidua* Mill. [Pinaceae], phytochemistry, phytotherapy, wound healing

1 INTRODUCTION

In advanced cancer patients, palliative care becomes the primary focus, in an attempt to alleviate the pain, treat the symptoms and improve the patient’s comfort (Vardhan et al., 2019). Among the most distressing discomforts that such patients have to endure, malignant fungating wounds account for a prevalence of 5%–14%. Malignant fungating wounds occur due to an aggressive proliferation and infiltration caused by a local tumor or a metastatic spread into the skin, blood and lymph vessels, resulting in tissue damage, hypoxia, necrosis, microbial proliferation and fungating ulceration of the wound. They are commonly present in the following body areas: breast (66%), head and neck (24%),
The effects of such wounds, also known as ulcerating wounds, malignant wounds or neoplastic lesions, cause physiological and psychological distress to the patients by affecting not only their wellbeing but also their social life. With respect to social life, it is known that due to the repellent malodor and the presence of exudates, patients are ashamed and therefore try to avoid social contact. This self-isolation leads to additional suffering and depression. At the same time, the treatment of these wounds remains a challenge (Regan, 2007; Adderley and Holt, 2014; Vardhan et al., 2019). Currently, options are limited and include the systemic and/or topical treatment of these wounds, followed by the groin, genital and back (3%), and various tissues (8%) (Tsichlakidou et al., 2019; Vardhan et al., 2019; Tilley et al., 2020). In addition, they are characterised by presenting a malodour, exudates, bleeding, pain, itching, irritation, infection, and necrosis (Adderley and Holt, 2014; Vardhan et al., 2019). The effects of such wounds, also known as ulcerating wounds, malignant wounds or neoplastic lesions, cause physiological and psychological distress to the patients by affecting not only their wellbeing but also their social life. With respect to social life, it is known that due to the repellent malodor and the presence of exudates, patients are ashamed and therefore try to avoid social contact. This self-isolation leads to additional suffering and depression. At the same time, the treatment of these wounds remains a challenge (Regan, 2007; Adderley and Holt, 2014; Vardhan et al., 2019). Currently, options are limited and include the systemic and/or topical application of analgesics, antibiotics, and coagulants (Regan, 2007; Adderley and Holt, 2014).

Ethnobotanical studies in the Balkan region described the use of L. decidua bark, needles and resin for internal and external use, for blood purification, renal, urinary, and gallbladder stones, in addition to wound healing, ulcers, and restlessness treatment (Saric-Kundalic et al., 2011; Jarić et al., 2018). The Committee for Veterinary Medicinal Products from the European Medicines Agency approved L. decidua resin for topical application in animals. The concentration of the resin varies from 10% to 20% for the treatment of skin wounds and promotion of wound healing (EMA, 1998). The German Drugbase lists it as an external application for rheumatic and neuralgic disorders, also for catarrhal illness in humans (Drugbase, 2021). A prospective, randomized and controlled multicenter trial using resin from Picea abies (L.) H.Karst. included 37 patients in the treatment of pressure ulcers and the healing activity was observed in 92% within the treated group (Sipponen et al., 2008). Sipponen et al. (2012) included 23 patients in their study and saw a healing rate of complicated chronic wounds of 100%, within a period of 43 ± 24 days. In addition, Goels et al. (2022) compared the wound healing potential of P. abies, Pinus nigra J.F.Arnold and L. decidua in vitro. The reduction of cell-free area in a keratinocyte wound healing assay was significant for the balm from L. decidua (26%) when compared to the P. abies balm and resin (16.7% and 9.6%, respectively) and to the P. nigra resin (16.2%). It is therefore the aim of the present review article to explore whether L. decidua’s resin (European larch tree), which has been used for wound healing for some time, as proposed in the context of anthroposophic medicine (Krüger, 1969), might offer new therapeutic options and therefore deserves to be investigated in more detail. Gaps in the existing knowledge were identified and addressed with respect to a systematic evaluation of in vitro and in vivo studies to justify the uses of this species, the standardized evaluation of pharmacological effects, limitations of existing studies, and prospects for future research and potential clinical applications.

2 MATERIALS AND METHODS

Literature search was performed using MEDLINE (PubMed), EMBASE and SciFinder databases. This scoping review aims at identifying the nature and extent of research evidence using systematic, transparent and replicable characteristics for data collection, analysis and interpretation and subsequently providing an overview or map of evidence on the topic (Grant and Booth, 2009; Munn et al., 2018). The phases implemented in this scoping review were: 1) collection of relevant literature; 2) selection of publications based on predefined criteria; 3) extraction of relevant data; 4) describing and synthesising the findings. There was no initial period or language restriction for the search. Literature covers a time period up to 26 March 2021. “Larix decidua” was used as a single search keyword. The inclusion criteria comprised: irrelevant outcome (genetic analysis, environmental behaviour, and wood properties), irrelevant sample (wood for construction, wood as furniture, and wood properties), insufficient data (results were not described for this species even though it was declared in the methods).
3 RESULTS

3.1 Identification of Studies

During the first phase of the literature search, \( n = 1,376 \) articles in English and \( n = 5 \) articles in German were identified. After a screening of the abstracts, 139 articles were assessed in more detail. After exclusion of 49 duplicates, \( n = 70 \) were considered to be eligible for a detailed full-text review after exclusion of studies with irrelevant outcome (\( n = 11 \)), irrelevant sample size (\( n = 6 \)), wrong species (\( n = 2 \)), or insufficient data (\( n = 1 \)). The 70 studies were categorized according to their field of research and/or outcome, such as \textit{in vivo}, \textit{ex vivo}, \textit{in vitro} with biological approach, \textit{in vitro} with chemical approach, \textit{in vitro} with biological and chemical approaches, and chemistry. 10 publications were excluded after reading the full text due to technical shortcomings or lack of critical information (Figure 1). A total of 60 publications were finally identified as satisfying the inclusion criteria for full article evaluation. The whole selection process is represented in Figure 1.

Included studies were published between 1952 and 2020, with 67% being published from 2001 onwards (Figure 2A). This demonstrates an increasing number of publications in later years and an increasing interest for the biological potential of \textit{L. decidua} over time and in particular since 2016. Figure 2B illustrates the listing of articles in different databases. Most studies were categorized as "chemistry" (\( n = 42 \)), followed by "\textit{in vitro} with biological and chemical approaches" (\( n = 7 \)), "\textit{in vitro} with biological approaches" (\( n = 6 \)), "\textit{in vivo}" (\( n = 2 \)), "\textit{in vitro} with chemical approaches" (\( n = 2 \)), and "\textit{ex vivo}" (\( n = 1 \)). These categories are in accordance to the higher number of publications found on SciFinder, which is a database for chemical literature.

3.2 Phytochemistry

The literature review showed that the majority of articles found for \textit{L. decidua} relates to its chemical composition. Amongst all the publications in this review (\( n = 60 \)), forty-two dealt with the chemical compounds found in different parts of the tree. Nine extra publications included the chemical analysis besides other \textit{in vitro} pharmacological investigations. The first study is dated from 1952 and is the oldest publication included in the review (Gripenberg, 1952). The most frequently tree parts used for extract preparation were: wood (\( n = 19 \)), bark (\( n = 17 \)), and needles (\( n = 16 \)), followed by resin (\( n = 8 \)), sawdust (\( n = 4 \)), and others (i.e., shoots, cone, branches, buds; \( n = 7 \)). Twenty-six studies (43%) did not mention the harvesting date, while 34 studies (57%) mentioned the period of harvesting or collection of the tree source. Twenty-six (43%) of 34 studies mentioned both month/season and year, while 8 mentioned only year or season or month. Eight studies (13%) did not mention the extractive solvent or the type of preparation of the used extracts in the study. Eleven studies (18%) did not mention the origin of the sample or its collection place, one sample was from non-European origin, and the remaining came from Europe (Table 1).

Table 2 shows the compounds that were described in at least two publications and/or those found in at least two different parts of the tree. To better show the chemical variety presented in \textit{L. decidua}, substances were categorized for different parts of the tree, the bark (\( n = 11 \)), the needles (\( n = 19 \)), the resin (\( n = 7 \)) and the wood (\( n = 19 \)). A total of 478 compounds were described for this tree (Supplementary Material), 118 are shown in Table 2. They were separated into different phytochemical categories, which included hydrocarbons (1), carbohydrates (2–9), flavonoids (10–18), terpenoids and their derivatives (19–90), fatty acids (91–100), other phenolic compounds (101–112), and others classes (113–118). Terpenoids and their derivatives were among the most common/most important class of compounds described for \textit{L. decidua}. Terpenoids and their derivatives in \textit{L. decidua} were composed of volatile terpenoids (mainly mono and sesquiterpenes) and non-volatile terpenoids (diterpenoids), depending on the part of the plant being investigated. The resin contains mainly diterpenoids and phenolic compounds, whilst the wood, needles, and bark present a more varied chemical composition. The most often

![Figure 2](image-url)
| Tree source | Extractive solvent | Collection/harvest period | Site of collection/ harvest | References |
|-------------|--------------------|---------------------------|-----------------------------|------------|
| Bark        | CH$_2$Cl$_2$       | nd                        | nd                          | Norin and Winell (1974) |
| Bark        | MeOH               | nd                        | nd                          | Matthews et al. (1997)  |
| Bark        | EtOAc              | September, 2008           | nd                          | Frederic et al. (2009)  |
| Bark        | MeOH               | September, 2012           | nd                          | Sgorlon et al. (2012)   |
| Bark        | Water (hot)        | March, 2012               | nd                          | Lareiter et al. (2014)  |
| Bark        | n-heptane, MeOH, MeOH:water | October, 2014 | nd                          | Bianchi et al. (2015)   |
| Bark        | EtOH               | 2017                      | nd                          | Hubert et al. (2016)    |
| Bark        | CH$_2$Cl$_2$, EtOAc, MeOH | 2009 and 2010 | nd                          | Baldan et al. (2017)    |
| Bark        | MeOH, water        | nd                        | nd                          | Mulholland et al. (2017) |
| Bark, resin (oleoresin) | CH$_2$Cl$_2$, ethyl acetate, MeOH (bark), n-hexane (turpentine) | December, 2013 | nd                          | Wagner et al. (2019)    |
| Bark, wood  | n-hexane           | August, 2014              | nd                          | Silero et al. (2020)    |
| Bark, wood  | MeOH               | February, 2015            | nd                          | Thuerig et al. (2018)   |
| Bark, wood  | Water (acidic) followed by diethyl ether addition (3x) | End of 2014 | nd                          | Czech Republic          |
| Branches    | nd                 | March, May, June, August, September, November 1976 and February 1977 | nd                          | Czech Republic          |
| Branches    | Hydrodistillation without solvent, followed by solubilization in n-hexane | nd | nd                          | Czech Republic          |
| Buds        | Glycerol/EtOH and water/glycerol/EtOH | February–April, 2018 and 2019 | nd                          | Czech Republic          |
| Cones       | Acetone, EtOH, MeOH | July–October, 2018        | nd                          | Czech Republic          |
| Essential oil (needles) | nd | June                        | nd                          | Czech Republic          |
| Essential oil (needles, wood) | nd | nd                         | nd                          | Czech Republic          |
| Essential oil (needles, wood, bark) | Hydrodistillation without solvent, followed by solubilization in n-pentane | nd | nd                          | Germany                 |
| Flower, cone | MeOH               | June, 1990                 | nd                          | Holm and Hiltunen (1997) |
| Leaves      | n-butanol, water (cold) | Autumn          | nd                          | King (1966)             |
| Leaves      | Acetone, EtOH       | Spring                     | nd                          | Good and Goodwin (1967) |
| Leaves, branches, stem, root | nd | November, 1981             | nd                          | Germany                 |
| Needles     | Water              | June–September             | nd                          | Lang and Messerer (1987) |
| Needles     | EtOH               | August, 1973               | nd                          | Austria                 |
| Needles     | Water              | October, December, January | nd                          | Nieminen and Bäas (1978) |
| Needles     | n-hexane           | July, November, December, 2003 | nd                          | Czech Republic          |
| Needles     | MeOH               | September, 2010            | nd                          | Switzerland             |
| Needles     | nd                 | May, 2013                  | nd                          | Switzerland             |
| Needles     | Water              | August, 2019               | nd                          | Poland                  |
| Needles (wax) | CHCl$_3$           | July, 1985                 | nd                          | Malá et al. (2013)      |
| Needles, shoots | Water (acidic)      | May, July, October, 2011   | nd                          | Switzerland             |
| Needles, twigs, bark, wood, trunk | Hexane, MeOH, water | January–March, 2018        | nd                          | Poland                  |
| Oleoresin   | Water (alkaline)   | July, 1985                 | nd                          | Dziedzinski et al. (2020)|
| Oleoresin   | Diethyl ether, water (alkaline) | July, 1985 | nd                          | Germany                 |
| Resin (callus resin, oleoresin) | EtOH       | 2003–2007                  | nd                          | Romaniascu et al. (2013)|
| Resin (oleoresin) | CH$_2$Cl$_2$      | nd                        | nd                          | Switzerland             |
| Resin (oleoresin) | Ether            | nd                        | nd                          | Finland                 |
| Resin, turpentine, essential oil | DMSO     | nd                        | nd                          | Norin (1972)            |
| Sawdust     | EtOH, water        | nd                        | nd                          | Bowiskakova et al. (1987)|
| Sawdust     | EtOH, n-heptane, water | nd                        | nd                          | Bowiskakova et al. (1988)|
| Sawdust     | Chemically standardized | nd                        | nd                          | Holmborn et al. (2008)  |
| Turpentine  | nd                 | nd                        | nd                          | Austria                 |

(Continued on following page)
described carbohydrates—galactose (4), glucose (6); flavonoids—kaempferol (14), taxifolin (17); volatile terpenoids and their derivatives—3-carene (21), camphene (24), limonene (31), α/β-pinene (54/62), β-phellandrene (61); non-volatile terpenoids (diterpenoids)—13-epimanool (69), abietic acid (71), dehydroabiatic acid (74), larixol (80), larixyl acetate (81); fatty acids—oleic acid (97), palmitic acid (98); phenolic acids—caffeic acid (101), ferulic acid (104), p-coumaric acid (107); others—benzoic acid (114). The chemical structures of the 22 most often described compounds are shown in Figure 3.

Different analytical methodologies were used for the separation, isolation, structural elucidation or identification of these compounds, such as TLC (thin layer chromatography), HPLC (high performance liquid chromatography), GC (gas chromatography), NMR (nuclear magnetic resonance), FTIR (Fourier transform infrared spectroscopy), among others. The most often used technique was GC, coupled to a laser desorption/ionization-time of flight (MALDI-TOF) and FTIR—attenuated total reflectance (ATR-FTIR) techniques.

3.3 Biological In Vitro Studies

3.3.1 Antimicrobial Activity

Antibacterial effect of bark and wood discs, as well as their methanol extracts were tested against four different species of bacteria (Table 3) (Laireiter et al., 2014). Larch bark discs inhibited S. aureus growth, whilst the wood discs did not. The wood discs methanol extract did not show any inhibitory effect on S. aureus, in contrast to the bark sawdust methanol extract. The bark discs and extract presented inhibitory effects on S. aureus while wood discs and extracts did not, showing that the tree source is an important factor for biological effects of L. decidua (Laireiter et al., 2014). Vállimaa et al. (2007) evaluated the antimicrobial properties against bacteria and fungi (Table 3) of a hexane wood extract, followed by extraction with acetone/water (95:1 v/v), which showed an inhibition against S. infantis (11%), B. cereus (31%), C. albicans (32%) and S. cerevisiae (17%). Bark methanol and aqueous extracts were tested against 4 species of microorganisms (Table 3), by which only the methanol extract affected the growth of S. aureus with an inhibitory zone of 8.2 mm (Wagner et al., 2019). The authors attributed the activity to the presence of the flavonoid kaempferol and the stilbenoid astringin (Wagner et al., 2019). Three different bark extracts (n-heptane, methanol, and methanol/water 50:50 (v/v)) were tested against S. aureus, in which the methanol (++++), methanol/water (+++), and n-heptane (+) presented antibacterial activity in a descending way, respectively (Hubert et al., 2016). The activity was correlated to the presence of phenolic compounds (Hubert et al., 2016). These studies showed that the antimicrobial activity [minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC)] depends on the plant part used and the solvent, as shown by the different effects on several microorganisms (Salem et al., 2016). Wood and bark methanol extracts were tested against nine different bacteria and six different fungi (Table 3). The bark extract presented lower MIC (0.11 mg/ml) compared to that of wood (0.13 mg/ml), in addition the minimum bactericidal concentration (MBC) varied from 0.36–0.96 mg/ml and 0.33–1.1 mg/ml, for the bark and wood extracts, respectively (Salem et al., 2016). All cited studies showed a better antimicrobial activity when using bark extracts when compared to wood. Two studies evaluated the activity of different larch extracts and isolated compounds against the fungus Plasmopora viticola. The MIC to completely inhibit zoospore germination and/or activity of P. viticola was 23 μg/ml for a turpentine formulation, 6 and 14 μg/ml for larixyl acetate and larixol, respectively (Thuerig et al., 2018). The authors

### Table 3

Continued General overview over the 60 included articles in the review.

| Tree source | Extractive solvent | Collection/harvest period | Site of collection/ harvest | References |
|-------------|--------------------|--------------------------|----------------------------|------------|
| Wood        | Ether              | nd                       | nd                         | Weinges (1961) |
| Wood        | MeOH, water       | nd                       | New Zeland                 | Uprichard (1963) |
| Wood        | Ethyl acetate     | August, 2015             | France                     | Fu et al. (2018) |
| Wood        | EtOH:toluene      | nd                       | Austria                    | Mecca et al. (2018) |
| Wood        | n-hexane          | nd                       | Czech Republic             | Wagner et al. (2020) |
| Wood (heartwood) | Acetone, hexane | nd                       | nd                         | Gripenber (1952) |
| Wood (heartwood, sapwood) | nd | May, 2003 | France                     | Wajs et al. (2007) |
| Wood (knotwood) | Hexane, acetone:water | nd               | Finland                    | Wilföer et al. (2003) |
| Wood (knotwood) | Hexane           | nd                       | nd                         | Vallmaa et al. (2007) |
| Wood (sapwood) | Hexane            | nd                       | nd                         | Wilföer et al. (2005) |
| Wood (hearthwood) | nd                 | nd                       | Austria                    | Becker et al. (2010) |
| Wood (sawdust) | nd                 | nd                       | Poland                     | Kopania et al. (2012) |
| Wood (softwood) | EtOH             | nd                       | nd                         |                |

CHCl₃, chloroform; CH₂Cl₂, dichloromethane; EtOAc, ethyl acetate; EtOH, ethanol; MeOH, methanol; nd, not declared.
| Class                     | No  | Compound                  | Tree part | Identification and analytical method | References                                      |
|--------------------------|-----|---------------------------|-----------|--------------------------------------|------------------------------------------------|
| Hydrocarbonates          | 1   | Methyl-cyclohexane        | Bark, wood| GC-MS                                | Salem et al. (2015b)                            |
| Carbohydrates            | 2   | Arabinose                 | Bark, wood| HPLC-UV, MALDI-TOF MS, GC-FID, GC-MS, GC-MS, GC, ATR-FTIR, NMR 1H | Wiltfö r et al. (2005); Bianchi et al. (2015); Hochegger et al. (2019) |
|                          | 3   | Fructose                  | Bark, needle| HPLC-UV, MALDI-TOF MS, GC-MS, GC-MS | Isidorov et al. (2005); Bianchi et al. (2015) |
|                          | 4   | Galactose                 | Bark, needle, wood| HPLC-UV, MALDI-TOF MS, GC-MS, GC-MS, GC, ATR-FTIR, NMR 1H | Isidorov et al. (2005); Wiltfö r et al. (2005); Bianchi et al. (2015); Hochegger et al. (2019) |
|                          | 5   | Galacturonic acid         | Bark, wood| HPLC-UV, MALDI-TOF MS, GC-MS, GC-MS, GC, ATR-FTIR, NMR 1H | Wiltfö r et al. (2005); Bianchi et al. (2015) |
|                          | 6   | Glucose                   | Bark, needle, wood| HPLC-UV, MALDI-TOF MS, GC-MS, GC-MS, GC, ATR-FTIR, NMR 1H | Isidorov et al. (2005); Willfö r et al. (2005); Bianchi et al. (2015); Hochegger et al. (2019) |
|                          | 7   | Mannose                   | Bark, wood| HPLC-UV, MALDI-TOF MS, GC-MS, GC-MS, GC, ATR-FTIR, NMR 1H | Wiltfö r et al. (2005); Bianchi et al. (2015); Hochegger et al. (2019) |
|                          | 8   | Sucrose                   | Bark      | HPLC-UV, MALDI-TOF MS, GC-MS, GC, ATR-FTIR, NMR 1H | Willfö r et al. (2005); Bianchi et al. (2015); Hochegger et al. (2019) |
|                          | 9   | Xylose                    | Wood      | GC-FID, GC-MS, GC, ATR-FTIR, NMR 1H | Willfö r et al. (2005); Bianchi et al. (2015); Hochegger et al. (2019) |
| Flavonoids               | 10  | Apigenin                  | Needle    | UPLC, UV, TLC | Niemann and Baas, (1978); Dzedzinski et al. (2020) |
|                          | 11  | Catechin                  | Bark, needle| HPLC-DAD-MS, HPLC-DAD, HPLC-FLD-MS, UV-Vis | Baldan et al. (2017); Turini et al. (2020) |
|                          | 12  | Dihydrokaempferol         | Wood      | TLC, GC-MS | Gripenberg (1952); Wiltfö r et al. (2003) |
|                          | 13  | Epicatechin               | Bark, needle| HPLC-DAD-MS, HPLC-DAD, HPLC-FLD-MS, UV-Vis | Baldan et al. (2017); Turini et al. (2020) |
|                          | 14  | Kaempferol                | Needle, wood| UV, TLC, GC-MS, FT-RAMAN, FT-IR, FT-NIR, UPLC | Niemann and Baas (1978); Dzedzinski et al. (2020); Wagner et al. (2020) |
|                          | 15  | Luteolin                  | Bark, needle| HPLC-DAD-MS, HPLC-FLD-MS, UPLC | Baldan et al. (2017); Dzedzinski et al. (2020) |
|                          | 16  | Quercetin                 | Needle, wood| HPLC-DAD-UV-Vis, UPLC | Dzedzinski et al. (2020); Turini et al. (2020) |
|                          | 17  | Taxifolin                 | Bark, wood| GC-MS, FT-RAMAN, FT-IR, FT-NIR, TLC | Gripenberg (1952); Norin (1972); Wagner et al. (2019); Wagner et al. (2020) |
|                          | 18  | Vitexin                   | Needle    | UV, TLC, UPLC | Niemann and Baas (1978); Dzedzinski et al. (2020) |
| Volatile Terpenoids      | 19  | (E/Z)-β-farnesene         | Needle, wood| GC-FID, GC-MS, NMR | Wajs et al. (2007); Garcia et al. (2017) |
|                          | 20  | 1,8-cineole               | Bark, needle| GC-FID, GC-MS | Kubeczka and Schultz (1987) |
|                          | 21  | 3-carene                  | Bark, needle| GC-FID, GC-MS, GC, NMR | Kubeczka and Schultz (1987); Lang and Messerer (1987); Lang (1989); Holm and Hiltunen (1997); Isidorov et al. (2005); Garcia et al. (2017) |
|                          | 22  | 4-terpinenol              | Wood      | GC-FID, GC-MS, NMR | Kubeczka and Schultz (1987); Salem et al. (2015b); Garcia et al. (2017) |
|                          | 23  | Bornyl acetate            | Bark, needle, wood| GC-FID, GC-MS, GC, NMR | Kubeczka and Schultz (1987); Isidorov et al. (2005); Wajs et al. (2007); Garcia et al. (2017) |
|                          | 24  | Camphene                  | Bark, needle, wood| GC-MS, GC-MS, GC-MS, GC, NMR | Kubeczka and Schultz (1987); Lang and Messerer (1987); Lang (1989); Holm and Hiltunen (1997); Wajs et al. (2007); Salem et al. (2015b); Garcia et al. (2017) |
|                          | 25  | Caryophyllene oxide       | Bark, needle, wood| GC-FID, HC-ICR ESI/APPI, GC, GC-MS, GC-MS, GC, NMR | Kubeczka and Schultz (1987); Isidorov et al. (2005); Garcia et al. (2017); Mofkoya et al. (2020) |
|                          | 26  | Cycloartenol              | Needle, wood| GC-FID, HPLC-Q-ToF-MS | Good and Goodwin (1967); Fu et al. (2018) |
|                          | 27  | Fenchol                   | Needle, wood| GC-FID, GC-MS, NMR | Salem et al. (2015b); Garcia et al. (2017) |
|                          | 28  | (Germanac-110)E,SE- den-4-ol | Needle, wood| GC-FID, GC-MS | Kubeczka and Schultz (1987) |
|                          | 29  | Germanacrene B            | Bark, needle, wood| GC-FID, GC-MS | Kubeczka and Schultz (1987); Wajs et al. (2007) |
|                          | 30  | Germanacrene D            | Bark, needle, wood| GC-FID, GC-MS, GC-MS, GC, NMR | Kubeczka and Schultz (1987); Wajs et al. (2007); Garcia et al. (2017); Mofkoya et al. (2020) |
|                          | 31  | Limonene                  | Bark, needle, wood| GC-FID, GC-MS, GC, NMR | Kubeczka and Schultz (1987); Lang and Messerer (1987); Holm and Hiltunen (1997); Isidorov et al. (2005); Wajs et al. (2007); Salem et al. (2015b); Garcia et al. (2017) |
|                          | 32  | Methyl thymol             | Needle, wood| GC-FID, GC-MS, GC-MS, GC, NMR | Wajs et al. (2007); Mofkoya et al. (2020) |
|                          | 33  | Myrcene                   | Bark, needle, wood| GC-FID, GC-MS, GC-MS, GC, NMR | Kubeczka and Schultz (1987); Lang and Messerer (1987); Holm and Hiltunen (1997); Wajs et al. (2007); Garcia et al. (2017) |

(Continued on following page)
TABLE 2 | (Continued) Chemical data of the 118 most important identified compounds from Larix decidua Mill. [Pinaceae], organized by chemical class, tree part, identification and analytical method. Abbreviations described in Section 3.2.

| Class | No | Compound | Tree part | Identification and analytical method | References |
|-------|----|----------|-----------|--------------------------------------|------------|
|       | 34 | Myrtenal | Bark, wood | GC-FID, GC-MS, NMR | Kubeczka and Schultz (1987); Garcia et al. (2017) |
|       | 35 | Myrtenol | Bark, wood | GC-FID, GC-MS, NMR | Kubeczka and Schultz (1987); Garcia et al. (2017) |
|       | 36 | β-cymen-8-ol | Needle, wood | GC-FID, GC-MS, NMR | Kubeczka and Schultz (1987); Lang and Messerer (1987); Holm and Hiltunen (1997); Wajs et al. (2007); Garcia et al. (2017) |
|       | 37 | β-cymene | Bark, needle, wood | GC-FID, GC-MS, NMR | Kubeczka and Schultz (1987); Holm and Hiltunen (1997); Garcia et al. (2017) |
|       | 38 | Pinocarvone | Bark, wood | GC-FID, GC-MS, NMR | Kubeczka and Schultz (1987); Garcia et al. (2017) |
|       | 39 | Sabinene | Bark, needle, wood | GC-FID, GC-MS | Kubeczka and Schultz (1987); Holm and Hiltunen (1997) |
|       | 40 | T-cadinol | Bark, needle, wood | GC-FID, GC-MS, NMR | Kubeczka and Schultz (1987); Wajs et al. (2007); Garcia et al. (2017); Bajer et al. (2020) |
|       | 41 | Terpinene-4-ol | Bark, needle | FT-ICR ESI/APPI, GC-FID, GC-MS | Kubeczka and Schultz (1987); Lang and Messerer (1987); Holm and Hiltunen (1997); Wajs et al. (2007); Garcia et al. (2017) |
|       | 42 | Terpinolene | Bark, needle, wood | GC-FID, GC-MS, NMR | Garcia et al. (2017); Mofkoya et al. (2020) |
|       | 43 | Thymol methyl ether | Needle, wood | FT-ICR ESI/APPI, GC-FID, GC-MS, NMR | Garcia et al. (2017); Mofkoya et al. (2020) |
|       | 44 | T-muurolol | Bark, needle, wood | GC-FID, GC-MS, NMR | Kubeczka and Schultz (1987); Garcia et al. (2017) |
|       | 45 | Trans-pinocarveol | Needle, wood | GC-FID, GC-MS, NMR | Garcia et al. (2017) |
|       | 46 | Trans-verbenol | Bark, wood | GC-FID, GC-MS, NMR | Kubeczka and Schultz (1987); Garcia et al. (2017) |
|       | 47 | Trieyelene | Bark, needle, wood | GC-FID, GC-MS | Kubeczka and Schultz (1987) |
|       | 48 | Verbenene | Bark, needle | GC-FID, GC-MS, FT-ICR ESI/APPI | Kubeczka and Schultz (1987); Mofkoya et al. (2020) |
|       | 49 | Verbenone | Needle, wood | GC-FID, GC-MS, NMR, FT-ICR ESI/APPI | Garcia et al. (2017); Bajer et al. (2020) |
|       | 50 | α-cadinol | Bark, needle, wood | GC-FID, GC-MS, NMR | Kubeczka and Schultz (1987); Garcia et al. (2017) |
|       | 51 | α-humulene | Bark, needle, wood | GC-FID, GC-MS, NMR | Kubeczka and Schultz (1987); Garcia et al. (2017) |
|       | 52 | α-murolene | Needle, wood | GC-FID, GC-MS, NMR | Kubeczka and Schultz (1987); Wajs et al. (2007); Garcia et al. (2017); Bajer et al. (2020) |
|       | 53 | α-phellandrene | Bark, needle, wood | GC-FID, GC-MS, NMR | Kubeczka and Schultz (1987); Holm and Hiltunen (1997); Wajs et al. (2007); Garcia et al. (2017) |
|       | 54 | α-pinene | Bark, needle, wood | GC-FID, GC-MS, GC, NMR | Kubeczka and Schultz (1987); Holm and Hiltunen (1997); Isidorov et al. (2005); Wajs et al. (2007); Garcia et al. (2017) |
|       | 55 | α-terpinene | Bark, needle, wood | GC-FID, GC-MS | Kubeczka and Schultz (1987); Lang and Messerer (1987); Holm and Hiltunen (1997); Wajs et al. (2007); Garcia et al. (2017) |
|       | 56 | α-terpineol | Bark, needle, wood | GC-FID, GC-MS, NMR | Kubeczka and Schultz (1987); Holm and Hiltunen (1997); Wajs et al. (2007); Garcia et al. (2017) |
|       | 57 | α-terpinyl acetate | Bark, wood | GC-FID, GC-MS | Kubeczka and Schultz (1987); Lang and Messerer (1987); Holm and Hiltunen (1997); Isidorov et al. (2005); Wajs et al. (2007); Garcia et al. (2017) |
|       | 58 | α-thujene | Needle, wood | GC-MS | Holm and Hiltunen (1997); Isidorov et al. (2005); Garcia et al. (2017) |
|       | 59 | β-carophyllene | Bark, needle, wood | GC-FID, GC-MS, GC, NMR | Kubeczka and Schultz (1987); Isidorov et al. (2005); Wajs et al. (2007); Garcia et al. (2017) |
|       | 60 | β-elemene | Needle, wood | GC-FID, GC-MS, NMR | Kubeczka and Schultz (1987); Holm and Hiltunen (1997); Isidorov et al. (2005); Wajs et al. (2007); Garcia et al. (2017) |
|       | 61 | β-phellandrene | Bark, needle, wood | GC-FID, GC-MS, GC, NMR | Kubeczka and Schultz (1987); Holm and Hiltunen (1997); Isidorov et al. (2005); Wajs et al. (2007); Garcia et al. (2017) |
|       | 62 | β-pinene | Bark, needle, wood | GC-FID, GC-MS, GC, NMR | Kubeczka and Schultz (1987); Holm and Hiltunen (1997); Isidorov et al. (2005); Wajs et al. (2007); Garcia et al. (2017) |
|       | 63 | γ-cadinene | Bark, needle, wood | GC-FID, GC-MS, NMR | Kubeczka and Schultz (1987); Holm and Hiltunen (1997); Isidorov et al. (2005); Wajs et al. (2007); Garcia et al. (2017) |
|       | 64 | γ-murolene | Needle | GC-FID, GC-MS, GC, NMR | Kubeczka and Schultz (1987); Holm and Hiltunen (1997); Isidorov et al. (2005); Wajs et al. (2007); Garcia et al. (2017) |
|       | 65 | γ-terpinene | Bark, needle, wood | GC-FID, GC-MS | Kubeczka and Schultz (1987); Holm and Hiltunen (1997); Isidorov et al. (2005); Wajs et al. (2007); Garcia et al. (2017) |
|       | 66 | δ-3-carene | Wood | GC-FID, GC-MS | Kubeczka and Schultz (1987); Wajs et al. (2007); Garcia et al. (2017) |
|       | 67 | δ-cadinene | Bark, needle | GC-FID, GC-MS | Kubeczka and Schultz (1987); Wajs et al. (2007); Garcia et al. (2017) |
|       | 68 | δ-cadinene | Needle, wood | GC-FID, GC-MS, NMR | Kubeczka and Schultz (1987); Wajs et al. (2007); Garcia et al. (2017) |

(Continued on following page)
### Table 2 (Continued) Chemical data of the 118 most important identified compounds from Larix decidua Mill. [Pinaceae], organized by chemical class, tree part, identification and analytical method. Abbreviations described in Section 3.2.

| Class | No | Compound | Tree part | Identification and analytical method | References |
|-------|----|----------|-----------|-------------------------------------|------------|
| **Non-Volatile Terpenoids (Diterpenoids)** | 69 | 13-epimanoool | Bark, resin, wood | IR, 1H/13C NMR, TLC, UV-Vis, GC, GC-FID, GC-MS | Norin (1972); Mills (1973); Norin and Winell (1974); Bol’shakova et al. (1988); Salem et al. (2015a); Thuerig et al. (2018); Dietemann et al. (2019); Garcia et al. (2017); Mofkoya et al. (2020) |
| 70 | Abietadiene | Needle, wood | FT-ICR ES/APPi, GC-FID, GC-MS, NMR | Mills (1973); Bol’shakova et al. (1987); Isidorov et al. (2005); Holmbom et al. (2008); Pferschy-Wenzig et al. (2008); Salem et al. (2018); Fu et al. (2018); Dietemann et al. (2019); Mofkoya et al. (2020) |
| 71 | Abietic acid | Bark, needle, resin, wood | IR, UV-Vis, NMR, GC, GC-FID, GC-MS, FT-ICR ES/APPi | Mills (1973); Bol’shakova et al. (1987); Isidorov et al. (2005); Holmbom et al. (2008); Pferschy-Wenzig et al. (2008); Dietemann et al. (2019); Mofkoya et al. (2020) |
| 72 | Abietol | Resin | GC-FID, GC-MS | Mills (1973); Holmbom et al. (2008) |
| 73 | Dehydroabietic acid | Needle, resin, wood | IR, UV-Vis, NMR, GC, GC-FID, GC-MS, FT-ICR ES/APPi | Mills (1973); Bol’shakova et al. (1987); Isidorov et al. (2005); Holmbom et al. (2008); Pferschy-Wenzig et al. (2008); Dietemann et al. (2019); Mofkoya et al. (2020) |
| 74 | Dehydroabietol | Resin, wood | GC-FID, GC-MS, NMR | Garcia et al. (2017) |
| 75 | Isopimaral | Bark, resin, wood | GC-FID, GC-MS | Mills (1973); Bol’shakova et al. (1987); Holmbom et al. (2008); Pferschy-Wenzig et al. (2008); Fu et al. (2018); Dietemann et al. (2019) |
| 76 | Isopimaric acid | Resin, wood | IR, UV-Vis, NMR, GC, GC-FID, GC-MS, HPLC-Q-ToF-MS | Norin (1972); Mills (1973); Bol’shakova et al. (1987); Holmbom et al. (2008); Willför et al. (2003); Holmbom et al. (2008) |
| 77 | Isopimarinal | Resin, wood | UV-Vis, GC, GC-FID, GC-MS | Bol’shakova et al. (1988); Rider et al. (2023) |
| 78 | Isopimaranol | Needle, wood | FT-ICR ES/APPi, GC-FID, GC-MS | Waj et al. (2007); Garcia et al. (2017) |
| 79 | Lariciresinol | Resin, wood | GC-FID, GC-MS, NMR | Mills (1973); Bol’shakova et al. (1987); Holmbom et al. (2008) |
| 80 | Larixol | Bark, resin, wood | UV-Vis, GC, GC-FID, GC-MS, FT-RAMAN, FT-IR, FT-NIR, 1H/13C NMR | Bol’shakova et al. (1987); Holmbom et al. (2008); Willför et al. (2003); Holmbom et al. (2008) |
| 81 | Larixyl acetate | Bark, resin, wood | UV-Vis, GC, GC-FID, GC-MS, 1H/13C NMR, ESIMS, IR | Norin (1972); Mills (1973); Bol’shakova et al. (1988); Pferschy-Wenzig et al. (2008); Muholland et al. (2017); Thuerig et al. (2018); Dietemann et al. (2019) |
| 82 | Levoabietic acid | Resin | IR, UV-Vis, NMR, GC, GC-FID, GC-MS | Mills (1973); Bol’shakova et al. (1987); Holmbom et al. (2008) |
| 83 | Manool | Needle, wood | GC-FID, GC-MS, NMR | Mills (1973); Bol’shakova et al. (1987); Holmbom et al. (2008) |
| 84 | Neocassic acid | Resin | IR, UV-Vis, NMR, GC, GC-FID, GC-MS | Norin (1972); Mills (1973); Bol’shakova et al. (1987); Holmbom et al. (2008); Willför et al. (2003); Holmbom et al. (2008) |
| 85 | Palustriic acid | Resin, wood | IR, UV-Vis, NMR, GC, GC-FID, GC-MS | Mills (1973); Bol’shakova et al. (1987); Holmbom et al. (2008); Willför et al. (2003); Holmbom et al. (2008) |
| 86 | Palustrol | Resin | UV-Vis, GC, GC-FID, GC-MS | Bol’shakova et al. (1988); Holmbom et al. (2008) |
| 87 | Pimarsato | Resin | GC-FID, GC-MS | Mills (1973); Dietemann et al. (2019) |
| 88 | Pimaric acid | Needle, Resin | FT-ICR ES/APPi, GC, GC-MS, GC-FID | Isidorov et al. (2005); Holmbom et al. (2008); Mofkoya et al. (2020) |
| 89 | Sandaracopimaric acid | Resin | IR, UV-Vis, NMR, GC, GC-FID, GC-MS | Mills (1973); Bol’shakova et al. (1987); Holmbom et al. (2008); Pferschy-Wenzig et al. (2008); Dietemann et al. (2019) |
| 90 | Secoisolariciresinol | Resin, wood | GC-FID, GC-MS | Willför et al. (2003); Holmbom et al. (2008) |
| **Fatty acids** | 91 | Arachidic acid | Bark, wood | GC | Salem et al. (2015a) |
| 92 | Hexadecanoic acid | Needle, wood | GC, GC-MS | Isidorov et al. (2005); Mecca et al. (2018) |
| 93 | Linoleic acid | Bark, wood | IR, NMR, TLC, HPLC-Q-ToF-MS | Norin and Winell, (1974); Salem et al. (2015a) |
| 94 | Margaric acid | Bark, wood | GC | Salem et al. (2015a) |
| 95 | Myristic acid | Bark | IR, NMR, TLC, GC | Norin and Winell, (1974); Salem et al. (2015a) |
| 96 | Octadecanoic acid | Needle, wood | GC, GC-MS | Isidorov et al. (2005); Mecca et al. (2018) |
| 97 | Oleic acid | Bark, needle, wood | IR, NMR, TLC, GC, FT-ICR ES/APPi, GC, GC-MS, IR, NMR, TLC | Norin and Winell, (1974); Isidorov et al. (2005); Mecca et al. (2018); Mofkoya et al. (2020) |
| 98 | Palmitic acid | Bark, needle, wood | IR, NMR, TLC, GC, FT-ICR ES/APPi, HPLC-Q-ToF-MS | Salem et al. (2015a); Fu et al. (2018); Mofkoya et al. (2020) |
| 99 | Pentadecanoic acid | Bark, wood | GC, GC-MS | Salem et al. (2015a); Mecca et al. (2018) |
| 100 | Stearic acid | Bark, needle, wood | IR, NMR, TLC, GC, FT-ICR ES/APPi | Norin and Winell, (1974); Salem et al. (2015a); Mofkoya et al. (2020) |
| **Other phenolic compounds** | 101 | Caffeic acid | Needle, resin | HPLC-DAD, UV-Vis, GC-FID, GC-MS, HPLC, UPLC | Lindner and Grill (1978); Kutters and Sarink (1986); Holmbom et al. (2008); Malá et al. (2013); Dziedzinski et al. (2020); Turrini et al. (2020) |
| 102 | Chlorogenic acid | Needle | GC, HPLC, UPLC | Lindner and Grill (1978); Malá et al. (2013); Dziedzinski et al. (2020) |

(Continued on following page)
suggest that both compounds represent valid candidates for use as antifungal substances in organic vineyards thereby reducing the use of copper. Bark CH$_2$Cl$_2$ extract (1 mg/ml) presented high efficacy and the isolated compounds (larixol, larixyl acetate and lariciresinol) at the same concentration (1.0 mg/ml) were very efficient (between 90% and 100%) against grapevine downy mildew, whereby larixyl acetate was the most efficient, showing 70% of efficacy at 0.1 mg/ml. This was the first report of lariciresinol as an antifungal compound.

**TABLE 2** (Continued) Chemical data of the 118 most important identified compounds from *Larix decidua* Mill. [Pinaceae], organized by chemical class, tree part, identification and analytical method. Abbreviations described in Section 3.2.

| Class No | Compound | Tree part | Identification and analytical method | References |
|----------|----------|-----------|-------------------------------------|------------|
| 103      | Cinnamic acid | Needle | GC-FID, GC, UPLC | Lindner and Grill (1978); Kuiters and Sarink, (1988); Dziedzinski et al. (2020) |
| 104      | Ferulic acid | Needle, resin | GC-FID, GC-MS, GC, HPLC, UV, TLC, UPLC | Lindner and Grill (1978); Niemann and Baas (1978); Kuiters and Sarink, (1988); Holmbom et al. (2008); Malá et al. (2013); Dziedzinski et al. (2020) |
| 105      | Gallic acid | Needle | GC-FID, GC, HPLC, UPLC | Lindner and Grill (1978); Kuiters and Sarink, (1988); Malá et al. (2013); Dziedzinski et al. (2020) |
| 106      | Lariciresinol | Bark | GC-MS, NMR, ESIMS, IR | Muhoffland et al. (2017); Wagner et al. (2019) |
| 107      | 3-coumaric acid | Needle, resin | GC-FID, GC-MS, GC, HPLC, UV, TLC, UPLC | Lindner and Grill (1978); Niemann and Baas (1978); Kuiters and Sarink (1988); Holmbom et al. (2008); Malá et al. (2013); Dziedzinski et al. (2020) |
| 108      | p-hydroxy benzoic acid | Needle | GC-FID, HPLC, UV, TLC, UPLC | Niemann and Baas (1978); Kuiters and Sarink (1988); Malá et al. (2013); Dziedzinski et al. (2020) |
| 109      | Pinoreinol | Bark, resin | GC-FID, GC-MS | Lindner and Grill (1978); Malá et al. (2013); Dziedzinski et al. (2020) |
| 110      | Protocatechuic acid | Needle | HPLC, GC | Lindner and Grill (1978); Kuiters and Sarink, (1988); Dziedzinski et al. (2020) |
| 111      | Syringic acid | Needle | GC-FID, GC, UPLC | Lindner and Grill (1978); Kuiters and Sarink, (1988); Dziedzinski et al. (2020) |
| 112      | vanillic acid | Needle | GC-FID, GC, HPLC, UV, TLC, UPLC | Lindner and Grill (1978); Kuiters and Sarink, (1988); Malá et al. (2013); Dziedzinski et al. (2020) |
|          | Other compounds | | | |
| 113      | Ascorbic acid | Needle | GC, HPLC | Lindner and Grill (1978); Radulescu et al. (2013) |
| 114      | Benzoic acid | Needle | FT-ICR ESI/APPI, GC-FID, GC | Lindner and Grill (1978); Kuiters and Sarink, (1988); Molfikoya et al. (2020) |
| 115      | Citric acid | Needle | GC, GC-MS | Lindner and Grill (1978); Isidorov et al. (2005) |
| 116      | Quinic acid | Needle | FT-ICR ESI/APPI, GC | Lindner and Grill (1978); Molfikoya et al. (2020) |
| 117      | Salicylic acid | Needle | GC-FID, UPLC | Kuiters and Sarink (1988); Dziedzinski et al. (2020) |
| 118      | Succinic acid | Needle | GC, GC-MS | Lindner and Grill (1978); Isidorov et al. (2005) |

**FIGURE 3** Phytochemical classes and their selected compounds identified in *L. decidua* Mill [Pinaceae].
TABLE 3 | Biological in vitro studies with Larix decidua Mill. [Pinaceae].

| Type of investigation | Sample | Assay | Cell/microorganism/material | Results | Author |
|-----------------------|--------|-------|----------------------------|---------|--------|
| Antimicrobial         | MeOH bark and wood extracts | Agar-diffusion test | S. aureus, P. aeruginosa, E. faecium, B. subtilis | Larch bark discs inhibited the growth of S. aureus, as well as bark sawdust MeOH extract (25 and 50 µL). In contrast, wood discs and wood MeOH extract did not present any inhibitory activity. Concluded that bark compounds are responsible for the antimicrobial activity | Laireiter et al. (2014) |
|                       | MeOH and water bark extract | Agar diffusion test | S. aureus, E. col, P. aeruginosa, C. albicans | MeOH extract (25/50 µL) presented antimicrobial effect against S. aureus (8.2 mm) | Wagner et al. (2019) |
|                       | n-heptane, MeOH, MeOH: water bark extracts | Immersion bioautography method | S. aureus | MeOH and MeOH:water extracts displayed antibacterial activity | Hubert et al. (2016) |
|                       | MeOH bark and wood extracts | Antifungal activity by the microdilution method and spore suspension; antibacterial activity by the micro-dilution method | P. funiculosum, P. ochrochloron, A. niger, A. flavus, A. ochraceus, C. albicans, B. cereus, D. solani, E. coli, L. monocytogenes, M. flavus, P. aeruginosa, P. atrosepticum, P. carotovorum ssp. carotovorum, S. aureus | MIC and MFC values of wood extracts were higher than the bark. Wood extract showed the highest MIC and MFC for A. flavus, A. niger, P. funiculosum. Wood and bark extracts exhibited antibacterial activity against all bacteria, but the bark was higher [MIC (0.11–0.54 mg/ml) and MBC (0.36–0.96 mg/ml)] than the wood one [MIC (0.13–0.54 mg/ml) and MBC (0.33–1.1 mg/ml)] | Salem et al. (2016) |
|                       | Hexane wood sawdust | Growth inhibition test using broth subcultures; inhibition zones in fungal confluent growth | E. coli, S. infantis, P. fluorescens, B. cereus, S. aureus, L. monocytogenes, L. plantarum, C. albicans, S. cerevisiae | MIC100 of 4 µL of extract (10 mg extractives/mL) presented inhibitory effect against S. infantis (11%), B. cereus (31%), C. albicans (32%) and S. cerevisiae (17%) | Villamaa et al. (2007) |
|                       | Turpentine, isolated compounds | Antifungal inhibition germination and/or activity of zoospores [MIC100] | Plasmopara viticola | Larch turpentine extract presented MIC100 of 23 µg/ml, larixyl acetate 6 µg/ml, and larixol 14 µg/ml | Thuerig et al. (2018) |
|                       | CH2Cl2 bark extract, isolated compounds | Antifungal inhibition germination and/or activity of zoospores | Plasmopara viticola | Larch turpentine extract presented MIC100 of 23 µg/ml, larixyl acetate 6 µg/ml, and larixol 14 µg/ml | Thuerig et al. (2018) |
|                       | Water needle extract | Antibacterial and antifungal activity through growth inhibition zone | K. penumoniae, S. enteritidis, P. aeruginosa, A. baumannii, E. faecium, S. aureus, L. fermentum, C. butyricum, L. monocytogenes, B. coagulans, C. utilis, Aspergillus spp., Fusarum spp. | It was observed no selectivity of the EtOAc extract on the tested cell lines: LoVo (IC50 68 µg/ml), PC3 (IC50 52 µg/ml), U373 (IC50 56 µg/ml); but it presented interesting cytotoxicity | Frederick et al. (2009) |
|                       | EtOAc bark extract | MTT assay | Human colon metastatic cell (LoVo), human prostate metastatic cell (PC3), human glioblastoma astrocytoma (U373) | It was observed no selectivity of the EtOAc extract on the tested cell lines: LoVo (IC50 68 µg/ml), PC3 (IC50 52 µg/ml), U373 (IC50 56 µg/ml); but it presented interesting cytotoxicity | Frederick et al. (2009) |
|                       | Isolated compounds | MTT assay, PI assay | Human embryonic kidney (HEK) | Larch turpentine and Venice turpentine presented IC50 of 100 µM | Urban et al. (2016) |
| Other                 | Turpentine, resin, essential oil, | Metabolic/physiological activity; TRPC-inhibition by Ca2+ variation | Human embryonic kidney (HEK) | It was observed no selectivity of the EtOAc extract on the tested cell lines: LoVo (IC50 68 µg/ml), PC3 (IC50 52 µg/ml), U373 (IC50 56 µg/ml); but it presented interesting cytotoxicity | Frederick et al. (2009) |

(Continued on following page)
concerning the activity of larch extracts against plant pathogenic oomycetes, which counts as a renewable resource at low prices for a sustainable plant protection (Mulholland et al., 2017). Water extract of needles presented antimicrobial activity against microorganisms of Gram-positive and Gram-negative bacteria as well as mold and yeast, with the most prominent result for *L. fermentum*, *S. aureus*, *C. butyricum* and *B. coagulans* (inhibition zones of 13 ± 2, 11 ± 2, and 10 ± 1, 10 ± 2 mm, respectively), which was correlated with the presence of phenolic compounds (Dziedzinski et al., 2020).

3.3.2 Cytotoxicity
An ethyl acetate macerated bark extract was tested for its anticancer potential *in vitro*, against three different human...
cancer cell lines (PC3, U373, LoVo; Table 3). The crude extract was incubated for 72 h and the cell viability was evaluated by 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide (MTT). Human prostatic adenocarcinoma (PC3; IC\textsubscript{50} 52 µg/ml) was slightly more sensitive to the extract than the human glioblastoma (U373; IC\textsubscript{50} 56 µg/ml), and lastly human colorectal adenocarcinoma (LoVo; IC\textsubscript{50} 68 µg/ml) was the most resistant (Frederich et al., 2009). However, other tree extracts (C. betulifolia [LoVo: IC\textsubscript{50} 85 µg/ml], C. sativa [LoVo: IC\textsubscript{50} 76 µg/ml; PC3: IC\textsubscript{50} 96 µg/ml; U373: IC\textsubscript{50} 86 µg/ml], F. sylvatica [PC3: IC\textsubscript{50} 70 µg/ml], I. aquifolium [PC3: IC\textsubscript{50} 76 µg/ml], Q. petraea [PC3: IC\textsubscript{50} 69 µg/ml], Q. robur [LoVo: IC\textsubscript{50} 80 µg/ml; PC3: IC\textsubscript{50} 75 µg/ml], R. pseudoacacia [LoVo: IC\textsubscript{50} 77 µg/ml; PC3: IC\textsubscript{50} 69 µg/ml; U373: IC\textsubscript{50} 94 µg/ml]) presented lower inhibitory activity against these human cancer cells (Frederich et al., 2009). Two isolated compounds from larch, larixol and larixyl acetate, were incubated with human embryonic kidney cells (HEK293). The integrity of the cells using propidium iodide (PI) assay (after 10 min and 24 h of compounds incubation) and their cell viability by MTT (after 24 h of compounds incubation) were evaluated (Urban et al., 2016). Membrane integrity was maintained at the three concentrations tested (2.5, 5, 10 µM) and cell viability and proliferation were also unaffected by the two tested compounds (25 and 50 µM) (Urban et al., 2016).

### 3.3.3 Other In Vitro Assays

In order to investigate the activity of some natural products that could abrogate pathophysiological responses within pulmonary and renal diseases, Ca\textsuperscript{2+} measurement was assessed on HEK 293 cell line (Table 3) (Urban et al., 2016). Larch turpentine (IC\textsubscript{50} 13 mg/L) and Venice Turpentine (IC\textsubscript{50} 140 mg/L; a mixture of larch turpentine and colophony) blocked Ca\textsuperscript{2+} entry through TRPC6 channel in a dose dependent manner, whilst the larch essential oil presented no activity. The authors concluded that the biological activity is due to the presence of the non-volatile resiniferous compounds, larixol (IC\textsubscript{50} 2.04 µM) and larixyl acetate (IC\textsubscript{50} 0.58 µM) (Urban et al., 2016).

Investigation on different tree species extracts for their potential as dermo-cosmetics assayed the effect of three different extracts from larch bark on three skin enzymes: collagenase, elastase and tyrosinase (Table 3). The incubation period for the collagenase and tyrosinase assays was 10 min and for the elastase 30 min, and the concentration of the tested extracts varied for each assay, in a range of 60–300 µg/ml (Hubert et al., 2016). Methanol extract was the most potent, followed by the methanol:water (50:50 v/v), and the less active was the n-heptane extract, for all assays. Elastase inhibitory activity was higher than 80% and 70% for the methanol and methanol:water extracts (300 µg/ml), respectively. The same profile was observed for collagenase, in which the inhibitory activity was higher than 90% and 80% at 150 µg/ml. Tyrosinase inhibitory activity was lower, but presented 50% and 40% for the methanol and methanol:water extracts (300 µg/ml), respectively. These results showed the potential of the bark extract to keep the skin homeostasis, by avoiding degradation of skin proteins, and to slow down skin pigments production in melanocytes, mainly due to the presence of phenolic substances (Hubert et al., 2016).

Becker et al. (2010) investigated the competitive inhibition of GM1-binding sites for cholera enterotoxins (Table 3). Larch wood sawdust and arabinogalactan (isolated from larch wood) at 0.5, 5 and 50 mg/ml presented a dose-dependent inhibition of toxin binding to GM1. An interesting finding for the wood sawdust (50 mg/ml) was that even when the toxin was already bound to the receptor, it was able to inhibit (62%) the binding at the same proportion as the pre-treatment (64%) or the simultaneous application of extract and toxin (62%). In contrast, arabinogalactan added after the toxin was already bound presented a very low interfering effect (15%) (Becker et al., 2010).

The influence of larch sawdust extracts on arachidonic acid cascade, a pro-inflammatory pathway, was evaluated in order to discover bioactive constituents from food, pharmaceutical and agricultural industries’ waste (Table 3) (Pferschy-Wenzig et al., 2008). Water, ethanol 70% and n-heptane extracts were prepared and then lyophilized. For the experiments, the dried samples were dissolved in absolute ethanol at a final concentration of 20 µg/ml. The n-heptane extract possessed pronounced anti-inflammatory activity, followed by the ethanol 70% extract and the water extract. The IC\textsubscript{50} values were 5 µg/ml, 0.1 µg/ml, and 11.1 µg/ml for COX-1, COX-2, and LTB\textsubscript{4}, respectively, for the n-heptane extract, while for the ethanol 70% extract it was 0.8 µg/ml for COX-2. To discover the active compounds, isolation of different chemicals from the n-heptane extract was carried out. The isolated diterpenes (Table 3) had inhibitory activity for LTB\textsubscript{4}, but only two presented inhibitory activity for COX-2, and none for COX-1. The authors inferred that other compounds than the isolated diterpenes must be responsible for the crude n-heptane extract inhibitory activity on COX-1 and COX-2, such as fatty acids, and that a series of diterpene acids were selective inhibitors of LTB\textsubscript{4} (Pferschy-Wenzig et al., 2008).

### 3.4 In Vivo Studies

Two studies evaluated standardized larch sawdust as ruminants’ dietary complement in comparison to controls (Table 4) (Sgorlon et al., 2012; Tedesco et al., 2015). Investigation of supplementation in 24 dairy cows in mid-lactation evaluated the effects on blood parameters and milk composition (Tedesco et al., 2015). The manufacturer standardized it by its content in fibre, protein, fat, ash, and lignin, whilst the group evaluated it through HPLC, standardizing it as 0.7% of taxifolin and 0.7% of dihydrokaempferol. It was given at a concentration of 300 g/day/cow, for 20 days, twice a day, and the milk parameters were evaluated at days 0, 7, 14 and 20, while blood parameters were just measured at days 0 and 20. No effect on milk parameters was identified, in contrast to urea, bilirubin, cholesterol, and VLDL concentration, which decreased in the blood, suggesting liver improvement, probably due to the presence of taxifolin, a compound that acts like statins and has antioxidant activity, contributing to hepatoprotection (Tedesco et al., 2015). Taxifolin was described in the bark, wood and the resin, making them sources to obtain this promising compound (Gripenberg, 1952; Norin, 1972; Wagner et al., 2019; Wagner et al., 2020). Sgorlon et al. (2012) evaluated larch sawdust counteraction on gene expression in blood leukocytes after
ACTH (adenocorticotropic hormone)-induced cortisol of thirty-six Sarda sheep. The amount of 50 g/head (5% of dry matter intake), which contained larixyl acetate and arabinogalactan as bioactive molecules, was given to the animals 15 days before treatment with ACTH. Cortisol concentration increased 8-fold for 3 and 51 h after ACTH treatment compared to the basal concentration, also increasing the down-regulation of transcripts up to 85.5% after 51 h. Larch sawdust supplementation regulated genes responsive to stress (GPX7, GADD45B, XRCC6, WRN1P1), to cell death pathways (NR4A1, GSK3B, TP53), to immune response (IFNG, MAPK3, NFKB1B) suggesting its use as an anti-inflammatory candidate for gene modulation (Sgorlon et al., 2012). The anti-inflammatory activity of larch sawdust was verified on sheep neutrophils (Farinacci et al., 2008) and against LTB4 and COX-2 formation (Pferschy-Wenzig et al., 2008), both studies in a different area of investigation but focused on biological anti-inflammatory activity.

### TABLE 4 | Studies about in vivo and ex vivo applications of Larix decidua Mill. [Pinaceae] derivatives.

| Type of investigation | Investigation | Sample | Biological source/animal model | Assay | Results | Author |
|-----------------------|--------------|--------|--------------------------------|-------|---------|--------|
| **In vivo**           | Effect of larch sawdust supplementation on blood parameters and milk composition | Chemically standardized sawdust | 24 multiparous Italian Friesian dairy cows in mid-lactation | 300 g of milled sawdust/day/cow | Milk parameters were unaffected. Blood metabolites were affected by larch sawdust intake. Blood urea concentration decreased, tendency for lowering glucose, total bilirubin decreased, and cholesterol tended to be lower than control. Concluded that larch improves liver function | Tedesco et al. (2015) |
| The effect of dietary administration on the modification of biological processes induced by high plasma cortisol | Chemically standardized sawdust | 36 Sarda sheep | 1 kg/head twice a day of basal diet, treating with 50 g/head of L. decidua Mill. [Pinaceae] bark 22 h before using twice a day with 0.5 ml of ACTH agonist (5 IM injections) | Cortisol concentration increased 8-fold greater than basal concentration (p < 0.001) with Larch use after ACTH treatment. After 51 h of ACTH and Larch bark treatment, down-regulation of transcripts increased (85.8%). Concluded that larch bark could be candidate as dietary supplements to modulate the modification of gene expression related to increased concentrations of cortisol | Sgorlon et al. (2012) |
| **Ex vivo**           | Evaluate the immunomodulatory activity of waste extracts on ovine neutrophils | EtOH 70% and water sawdust extracts | Ovine neutrophils from 8 healthy sheep | MTT viability assay; acid phosphatase adhesion assay; superoxide production assay by horse-heart ferricytochrome c | EtOH 70% (2.23–60 μg/ml) extract significantly reduced the MTT metabolism of neutrophils in a dose-dependent manner (>60%), whilst the aqueous (6.67–180 μg/ml) had no effect on neutrophil viability. The EtOH extract strongly blocked neutrophil adhesion (IC50 10.89 μg/ml) and inhibited the superoxide production from activated neutrophils (IC50 8.15 μg/ml) in a dose-dependent manner. Concluded that extract has anti-inflammatory activity on sheep neutrophils, possibly due to the presence of flavonoids and arabinogalactan | Farinacci et al. (2008) |
Unfortunately, abietic acid and abietanes are readily oxidized (Scalarone et al., 2002; Osete-Cortina and Domenech-Carbo, 2005). These oxidized products, such as 15-hydroperoxydehydroabietic acid, 15-hydroxyabietic acid methyl ester, 7-oxodehydroabietic acid methyl ester, are reported as responsible for contact allergy and dermatitis (Karlberg and Liden, 1985; Hausen et al., 1993; Downs and Sansom, 1999; Barchino-Ortiz et al., 2008). However, animal experiments could not substantiate this concern. *L. decidua* seems to be safe and well tolerated. In animal studies, oral intake of larch sawdust did not show any harm to cows or sheep (Tedesco et al., 2015). Each animal received 300 g of milled sawdust daily for 20 days, standardized with 0.7% of taxifolin and 0.7% of dihydrokaempferol. These two compounds are present in the bark (Wagner et al., 2019), resin (Norin, 1972) and wood (Gripenberg, 1952; Willför et al., 2003; Wagner et al., 2020). They have already been described in the literature as promising supplementary sources with anti-inflammatory, anticancer, antioxidant, and hepatoprotective activities (Kashyap et al., 2017; Sunil and Xu, 2019). Thus, larch industrial waste product can be used as animal supplements with no indications of adverse effects on the wellbeing of the exposed animals. It is our impression that topical applications of plant extracts and resin, as needed for the treatment of wounds, should be safe and well tolerated. We understand that mild adverse effects such as contact dermatitis are transient, can be easily

**TABLE 5 | Antioxidant evaluation of *Larix decidua* Mill. [Pinaceae] extracts.**

| Analytical method                      | Results                                                                 | Authors                        |
|----------------------------------------|-------------------------------------------------------------------------|--------------------------------|
| Total Phenolic Content (TPC)           | Acetone: water 80:20 v/v extract: green cones (73.55 ± 4.11 mg GAE/g dw), mature cones (26.90 ± 5.79 mg GAE/g dw), opened cones (16.84 ± 0.90 mg GAE/g dw) | Hofmann et al. (2020)          |
|                                        | MeOH:water 80:20 v/v extract: Green cones (49.40 ± 0.82 mg GAE/g dw), mature cones (14.48 ± 1.95 mg GAE/g dw), opened cones (13.13 ± 0.75 mg GAE/g dw) |                                |
|                                        | EtOH:water 80:20 v/v extract: Green cones (43.63 ± 0.38 mg GAE/g dw), mature cones (7.49 ± 0.55 mg GAE/g dw), opened cones (10.97 ± 0.09 mg GAE/g dw) |                                |
|                                        | Bark: EtOH:water 50%/50% (538 mg GAE/g dw)                               | Sillero et al. (2020)          |
|                                        | Bark: water extract (16.47% ± 0.52%); EtOH 40% (20.19% ± 1.59%); EtOH 60% (34.28% ± 0.37%); EtOH 80% (29.85% ± 0.30%) (w/w rutin) |                                |
|                                        | Bark water extract (46.7 mg epicatechin/kg dw)                           |                                |
|                                        | Needle water extract (14.83 ± 0.30 mg GAE/g dw)                          |                                |
| DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging assay | Acetone: water 80:20 v/v extract: green cones (IC<sub>50</sub> 13.73 ± 1.30 µg/ml), mature cones (IC<sub>50</sub> 12.27 ± 1.14 µg/ml), opened cones (IC<sub>50</sub> 14.39 ± 0.75 µg/ml) | Hofmann et al. (2020)          |
|                                        | Bark EtOH:water 50%/50% (636 mg TE/g dw)                                | Sillero et al. (2020)          |
|                                        | Bark MeOH extract (>90% GAE)                                            | Hubert et al. (2016)           |
|                                        | MeOH extract: heartwood (80%), sapwood (70%), knotwood (90%), bark (90%) | Piccand et al. (2019)          |
|                                        | Water extract: heartwood (20%), sapwood (1%), knotwood (10%), bark (90%) (GAE) |                                |
|                                        | Bark EtOH 40% extract (3.93 ± 0.38 µg/ml)                                | Baldan et al. (2017)           |
|                                        | Sawdust EtOH 75% v/v (9.9–15.6 µg/ml)                                   | Hochegger et al. (2019)        |
|                                        | Needle water extract (326.93 ± 21.21 µM Trolox/g dw)                     | Cesedinzki et al. (2020)       |
| Ferric reducing antioxidant power (FRAP) | Acetone: water 80:20 v/v extract: green cones (40.39 ± 0.73 mg AAE/g dw), opened cones (8.07 ± 0.46 mg AAE/g dw), mature cones (7.79 ± 0.52 mg AAE/g dw) | Hofmann et al. (2020)          |
|                                        | Bark EtOH:water 50%/50% (441 mg TE/g dw)                                | Sillero et al. (2020)          |
| Total Flavonoid Content (TFC)          | Bark EtOH:water 50%/50% (593 mg CE/g dw)                                | Sillero et al. (2020)          |
| ABTS                                  | Bark EtOH:water 50%/50% (1,040 mg CE/g dw)                              | Sillero et al. (2020)          |
| Lipid peroxidation inhibitory assay in rat liver microsomes in vitro; scavenging of peroxyl radicals by chemiluminescence | Wood hexane extract followed by acetone:water (95:5 v/v) extraction showed IC<sub>50</sub> value of 57 µg/L on inhibition of lipid peroxidation, 35 µg/L on scavenging of superoxide radicals, and 6.4 mmol/g on scavenging of peroxyl radicals | Willför et al. (2003)          |

GAE, gallic acid equivalents; TE, trolox equivalents; AAE, ascorbic acid equivalents; CE, catechin equivalents.
detected, and can be controlled by discontinuation of a confined topical exposure.

### 3.5 Ex Vivo Studies

Farinacci et al. (2008) carried out an ex vivo analysis with sawdust extracts on ovine neutrophils, which aimed to evaluate the immunomodulatory activity by MTT assay (Table 4). 70% ethanol extract [2.23–60 μg/ml] significantly reduced the metabolism of neutrophils in a dose-dependent manner (>60%), whilst the aqueous extract [6.67–180 μg/ml] presented no effect on neutrophil viability. Activated neutrophils chemotactically migrate to the site of infection or inflammation after firm adhesion to endothelial cells followed by transmigration, production of superoxides and respiratory burst, which this study attempted to verify. The 70% ethanol extract strongly blocked neutrophil adhesion (IC₅₀ 10.89 μg/ml) and inhibited the superoxide production from activated neutrophils (IC₅₀ 8.15 μg/ml), concluding that the extract had anti-inflammatory activity on sheep neutrophils, possible due to the presence of flavonoids and arabinogalactan (Farinacci et al., 2008). However, these effects do not seem to be independent of cytotoxic effects and cannot be perceived as an isolated anti-inflammatory action. As described by Pierschky-Wenzig et al. (2008), the anti-inflammatory activity could be ascribed to diterpene acids, such as larixyl acetate and palustric acid, which possess anti-inflammatory activity against COX-2.

### 3.6 Antioxidant Potential

Five spectrometric analytical methods were used to evaluate the antioxidant potential of different extracts derived from *L. decidua* (Table 5). Total phenolic content (TPC) was evaluated through the Folin-Ciocalteu test, which measures the reducing power of phenolic antioxidants, mainly using gallic acid and catechins as reference standards (Munteanu and Apetrei, 2021). The antioxidant activity of plant extracts is commonly assessed by 2,2-diphenyl-1-picryl-hydrazyl (DPPH) test, at a low cost, easy to perform and it is based on the transfer of electrons from the antioxidant source to the DPPH reagent and the result is often reported as EC₅₀ (Alam et al., 2013; Munteanu and Apetrei, 2021). The ferric reducing antioxidant power (FRAP) assay evaluates the ability of antioxidants to reduce ferric iron in acid pH conditions, by an increasing of absorbance (Alam et al., 2013; Munteanu and Apetrei, 2021). The trolox equivalent antioxidant capacity measures the total antioxidant capacity to neutralize the 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) stable cationic radical, in which antioxidants decreases the absorption intensity (Munteanu and Apetrei, 2021). The last method was the determination of total flavonoid content (TFC) by the aluminium chloride colorimetric assay, which is the most commonly applied assay for flavonoid determination in food and plant derivatives (Pekal and Pyrzynska, 2014).

Comparison of results was not easy to manage, as the investigations were performed using different tree parts, different extractive solvents and ratios, and different reference standards. It is important to point out that some of the studies themselves performed comparative investigation, either by the tree part or by the extractive solvent (Table 5). The TPC is higher in green cones (73.55 ± 4.11 mg GAE/g dw) when compared to mature (26.90 ± 5.79 mg GAE/g dw) or older ones (16.84 ± 0.90 mg GAE/g dw), and also in more polar extractive solvent, such as acetone-water. After this first finding, the authors followed the DPPH and FRAP assays only with the acetone-water extracts, which demonstrated an average similar pattern for all aged cones to the DPPH assay (green cones: IC₅₀ 13.73 ± 1.30; mature cones: IC₅₀ 12.27 ± 1.14; andopened cones: IC₅₀ 14.39 ± 0.75 μg/ml) but also a higher FRAP to the green cones (40.39 ± 0.73 mg AAE/g dw) compared to the mature (7.79 ± 0.52 mg AAE/g dw) and ripen ones (8.07 ± 0.46 mg AAE/g dw) (Hofmann et al., 2020). This publication demonstrates how important it is to investigate different extractive solvents as well as the tree parts and in different developmental stages, as an organ develops, it changes its composition and its chemical/biological outcomes. Another example is given by the DPPH assay, in which two extractive solvents (MeOH and water) were evaluated for different tree parts (heartwood, sapwood, knotwood, and bark). The MeOH extracts [heartwood (80%), sapwood (70%), knotwood (90%), bark (90%) (GAE)] were mainly more active when compared to the water extracts [heartwood (20%), sapwood (1%), knotwood (10%), bark (90%) (GAE)], and the tree parts resulted in different activities, mostly the bark as the most potent (Piccand et al., 2019).

The anti-oxidative potency of an acetone-water extract (2% v/v) was evaluated using rat liver microsomes in vitro (Table 5) (Willför et al., 2003). It presented IC₅₀ value of 57 μg/L on lipid peroxidation, while the tested control compounds, Trolox and butylated hydroxyanisole (BHA), presented IC₅₀ of 5 and 198 μg/L, respectively. The activity for scavenging of superoxide radicals was lower (IC₅₀ value of 35 μg/L) than the tested control compounds (BHA and Trolox, 2.7 and 6.3 μg/L, respectively). The trapping capacity by scavenging of peroxyl radicals was 6.4 mmol/g, higher than the one of the control Trolox (8 mmol/g). Concluding the potential as a source of natural antioxidant, mainly due to the synergistic effect of phenolic compounds, such as lignans, taxifolin and secoisolariciresinol (Willför et al., 2003).

### 4 DISCUSSION

This review resulted in a compilation of the main chemical constituents as well as the main pharmacological properties, in vitro and in vivo, described for the species *L. decidua*. As described before, European Larch resin is an oil resin, composed mainly of monoterpenes and diterpenes, among other classes of chemical compounds. Copaiba oil, an oil resin obtained from plants belonging to the genus *Copaifera*, is another example of such intricate mixtures of volatile terpenes and non-volatile terpenes (Tobouti et al., 2017; Cicek et al., 2018; Pfeifer Barbosa et al., 2019). It is therefore the aim of the following sections to present potential benefits of larch extracts for therapeutic applications. In particular, we follow up on the hypothesis that larch extracts might have a beneficial effect for...
the treatment of ulcerating wounds. Our key learnings can be summarized as follows:

### 4.1 Different Classes of Chemicals Contribute to the Observed Effects

Plant extracts contain a multitude of secondary metabolites. Chemical analysis detected a variety of chemical classes and provided an important piece of information for Larch (*L. decidua*). The most prevalent phytochemical class for each tree part can be summarized as follows. Bark: flavonoids, volatile terpenoids and fatty acids. Needles: flavonoids, volatile terpenoids and phenolic acids. Wood: volatile terpenoids, diterpenoids and fatty acids. Resin: diterpenoids and phenolic compounds. The class of terpenoids, especially the diterpenoids, has received most attention in studies, which have tested isolated compounds of defined chemical composition (Pferschy-Wenzig et al., 2008; Urban et al., 2016; Mulholland et al., 2017; Thuerig et al., 2018). The origins of the term terpene or terpenoid, the largest and most diverse class of plant metabolic compounds, comes from the German word turpentine—*Terpentīn*—from which the first compounds of this class were isolated and structurally determined (Langenheim, 2003). The term turpentine is unspecific and is used for different types of resins, but it is known that Venice turpentine, also called larch turpentine, is derived from *L. decidua* (Scalarone et al., 2002; Dietemann et al., 2019), which has a clear and light yellowish appearance (HAB, 2014; Dietemann et al., 2019; Drugbase, 2021). Resins can be described as a lipid-soluble mixture of volatile and non-volatile terpenoid and/or phenolic compounds (Table 2), which are preformed and stored in secretory structures or may be induced at the site of an injury (Langenheim, 2003). Nevertheless, the European medicines agency (EMA, 1998) and the German Drugbase database (Drugbase, 2021) describe the composition of resin with approximately 15% of essential oils (monoterpenoids) and 50%–65% of resin acids (diterpenoids) without mentioning other potential active pharmaceutical ingredients. Thus, while terpenoids may be considered to be marker compounds for chemical standardization, they are most likely not the sole constituents contributing to the observed pharmacological actions.

### 4.2 Anti-inflammatory Effects of Resin are Often Attributed to the Action of the Diterpene Larixyl Acetate

Diterpenes in conifer resins are characterized to contain three main structural types, being abietanes (levopimaric acid, abietic acid, neoabietic acid, etc), pimaranes (pimaric acid, sandaracopimaric acid, isopimaric acid, etc) and labdanes (epimanool, larixol, larixyl acetate, etc) (Mills and White, 1987; Scalarone et al., 2002; Langenheim, 2003). Abietic acid is present in all parts of the tree. Recent publications have described its biological potential to be anti-inflammatory (Gao et al., 2016; Kang et al., 2018; Thummuri et al., 2018). Several studies suggest that abietic acid may interfere with signalling pathways and cytokine homeostasis. This includes inhibition of NF-κB and MAPK signalling pathways and inhibition of NFATc1 and c-Fos (Thummuri et al., 2018). This view is supported by the *in vivo* attenuation of allergic asthma in mouse, which is possibly related to the inhibition of NF-κB activation (Gao et al., 2016). Kang et al. (2018) describe activation of PPAR-γ, suppression of IL-1β, and inhibition of release of TNF-α, NO, and PGE2 by abietic acid. Therefore, abietic acid might be a promising candidate for the treatment of inflammatory disease and, as a consequence, have positive effects on wound healing. This might be cooperative effects with other larch constituents, such as larixyl acetate (see below) or taxifolin (Kolhir et al., 1996).

### 4.3 Antimicrobial Effects of Resin are Often Attributed to the Action of the Diterpene Larixyl Acetate

Larixyl acetate is one of the most described diterpene present in *L. decidua* in the bark (Mulholland et al., 2017; Thuerig et al., 2018), wood (Pferschy-Wenzig et al., 2008; Thuerig et al., 2018), and resin (Norin, 1972; Mills, 1973; Bol’shakova et al., 1988; Dietemann et al., 2019). Antimicrobial activity of the isolated larixyl acetate was demonstrated against *P. viticola*, with MIC<sub>100</sub> of 6 µg/ml (Thuerig et al., 2018) and an efficacy of 100% at 1 mg/ml (Mulholland et al., 2017). It was therefore suggested to be effective against grapevine downy mildew, the most devastating pathogen of grapevines. It should be noted that larixyl acetate displays as well anti-inflammatory activity. These effects are mediated by inhibition of cyclooxygenase COX-2 and leukotriene LTB4 biosynthesis, with IC<sub>50</sub> values of 95.1 and 10.4 µM, respectively (Pferschy-Wenzig et al., 2008). In addition, larixyl acetate and arabinogalactan, supplied as dietary supplementation in the form of larch bark for 22 days, showed modulation of cortisol concentration in sheep (Sgorlon et al., 2012). We therefore propose that the confirmed antimicrobial and proposed anti-inflammatory effects of larixyl acetate might contribute in a positive way to wound healing (Tobouti et al., 2017).

### 4.4 Larch Arabinogalactan is a Dietary Fibre With Toxin-Binding and Protective Effects on Epithelia of Endodermal Origin

Larch arabinogalactan, a FDA-approved dietary fibre, has been described in the literature to possess several biological activities, such as gastrointestinal mucosal protection, improvement of the gut microflora, stimulation of the immune system, and inhibition of metastatic tumour cells of the liver (Kelly, 1999; Kim et al., 2002; Silvani et al., 2020). Acute and prolonged toxicity tests on rats demonstrated no evidence of toxicity at a single dose of 5,000 mg/kg or with 500 mg/kg daily during 90 days, respectively (Kelly, 1999). A study of particular interest compared different natural compounds and extracts for their preventive activity on cholera or travelers’ diarrhea (Becker et al., 2010). Larch arabinogalactan and *L. decidua* sawdust showed binding to GM1-binding sites of cholera toxin. Dietary intake led to dose dependent beneficial effects (Becker et al., 2010). We therefore propose that larch arabinogalactan might have the potential to...
absorb bacterial toxins and to prevent bacterial invasion of wounds.

5 CONCLUSION

Our review shows that there is an increasing interest in the use of *L. decidua* and in particular in questions related to the chemical composition of its extracts. Regrettably, there was in many cases missing information, such as collection site or time of harvesting. This is a major shortcoming since this information is required to keep the traceability of the provenance of the material and to describe chemical variability due to seasonal changes and site of collection. Ethnobiological observations and approved veterinary use shows a beneficial effect of topical applications of *L. decidua* resin on wound healing. Our literature review confirms this notion and provides supportive evidence, since extracts of *L. decidua* were shown to have anti-inflammatory, anti-infective, and tissue protective effects. However, these pharmacological activities cannot be attributed to the single action of a defined chemical entity but seem to be the result of a complex interplay between different compounds. More research in the field will be necessary for an understanding of the mechanisms by which this oil resin can be used to treat ulcerating wounds. For future work we propose a differentiated pharmacological investigation of the *L. decidua*’s different components, volatile and non-volatile fractions, separately, to ascertain which chemical compounds of the extracts are responsible for specific effects and to determine if synergistic effects are playing any role. The demonstrated safety and tolerability of *L. decidua* constituents’ warrants research in this field with the prospect for the implementation of new therapeutic applications.

AUTHOR CONTRIBUTIONS

JB, CH, JH, JM, and SB contributed to the review conception and design. Material preparation, data collection and analysis were performed by JB. Publications in German were evaluated by AU. FB contributed to the phytochemical part and to the final review of the paper. All the authors read, revised and approved the final article.

FUNDING

This review was funded by University of Bern.

ACKNOWLEDGMENTS

We acknowledge Konrad Urech for critical comments and inputs for this publication.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2022.895838/full#supplementary-material

REFERENCES

Adderley, U. J., and Holt, I. G. (2014). Topical Agents and Dressings for Fungating Wounds. *Cochrane Database Syst. Rev.* 5, CD003948. doi:10.1002/14651858.CD003948.pub3

Alam, M. N., Bristi, N. J., and Rafiquzzaman, M. (2013). Review on In Vivo and In Vitro Methods Evaluation of Antioxidant Activity. *Saudi Pharm. J.* 21 (2), 143–152. doi:10.1016/j.jsps.2012.05.002

Andersen, Ø. M. (1992). Anthocyanins from Reproductive Structures in Pinaceae. *Biochem. Syst. Ecol.* 20 (2), 145–148. doi:10.1016/0305-1978(92)90101-i

Bajer, T., Šuj, J., Ventura, K., and Bajerová, P. (2020). Volatile Compounds Fingerprinting of Larch Tree Samples for Siberian and European Larch Distinction. *Eur. J. Wood Prod.* 78 (2), 393–402. doi:10.1007/s00107-020-01498-w

Baldan, V., Sut, S., Faggian, M., Dalla Gassa, E., Ferrari, S., De Nadai, G., et al. (2017). *Larix Decidua* Bark as a Source of Phytoconstituents: An LC-MS Study. *Molecules* 22 (11), 1974. doi:10.3390/molecules22111974

Barchino-Ortiz, L., Cabeza-Martínez, R., Leis-Dosil, V. M., Suárez-Fernández, R. M., and Lázaro-Ochaita, P. (2008). Allergic Contact Hypersensitivity from Turpentine. *Allergol. Immunopathol. Madrid.* 36 (2), 117–119. doi:10.1157/13120411

Becker, P. M., Widjaja-Greeffes, H. C., and van Wikselaar, P. G. (2010). Inhibition of Binding of the AB5-type Enterotoxins LT-I and Cholera Toxin to Ganglioside GM1 by Galactose-Rich Dietary Components. *Foodborne Pathog. Dis.* 7 (3), 225–233. doi:10.1089/fpd.2009.0387

Bianchi, S., Krosłakowa, I., Janzon, R., Mayer, I., Saake, B., and Pichelin, F. (2015). Characterization of Condensed Tannins and Carbohydrates in Hot Water Bark Extracts of European Softwood Species. *Phytochemistry* 120, 53–61. doi:10.1016/j.phytochem.2015.10.006

Boł’shakova, V. I., Demenková, L. I., Schmidt, N., and Pentegová, V. A. (1987). Resin Acids of the Oleoresins of Conifers Growing in Transcarpathia. *Chem. Nat. Compd.* 23, 173–175. doi:10.1007/BF00598751

Boł’shakova, V. I., Demenková, L. I., Schmidt, E. N., and Pentegová, V. A. (1988). Neutral Diterpenoids of Oleoresins of Five Species of Conifers of Transcarpathia. *Chem. Nat. Compd.* 24 (6), 691–694. doi:10.1007/BF00598185

Churakova Sidorova, O. V., Lehmann, M. M., Siegwolf, R. T. W., Saurer, M., Fonti, M. V., Schmidt, L., et al. (2019). Compound-specific Carbon Isotope Patterns in Needles of Conifer Tree Species from the Swiss National Park under Recent Climate Change. *Plant Physiol. Biochem.* 139, 264–272. doi:10.1016/j.plaphy.2019.03.016

Ciçek, S. S., Pfeifer Barbosa, A. L., and Girreser, U. (2018). Quantification of Diterpene Acids in Copaiba Oleoresin by UHPLC-ELSD and Heteronuclear Two-Dimensional qNMR. *J. Pharm. Biomed. Analysis* 160, 126–134. doi:10.1016/j.jpba.2018.07.034

Dietemann, P., Miller, K. v., Höpker, C., and Baumer, U. (2019). On the Use and Differentiation of Resins from Pinaceae Species in European Artworks Based on Written Sources, Reconstructions and Analysis. *Stud. Conservation 64* (Suppl. 1), S62–S73. doi:10.1080/00393630.2019.1568678

Downs, A. M., and Sansom, J. E. (1999). Colophony Allergy: a Review. *Contact Dermat.* 41 (6), 305–310. doi:10.1111/j.1600-0536.1999.tb00178.x

Drugbase (2021). *Terebinthina Laricina* [Online]. Available: https://www.drugbase.de/de/datenbanken/hagers-enzyklopaedie/artikel.html?tx_crondavdbhager_pi%5Buid%5D=55406&cHash=cb1c3209ad35a33d5c80ad879e52401 (Accessed August 15, 2021).

Dziędzinski, M., Kobus-Cisowska, J., Szymanowska, D., Stuper-Szablewska, K., and Baranowska, M. (2020). Identification of Polyphenols from Coniferous Shoots as Natural Antioxidants and Antimicrobial Compounds. *Molecules* 25 (15), 3527. doi:10.3390/molecules25153527
EMA (1998). *Terebinthinae Laricina EMAE/MRL/398/98: Committee for Veterinary Medicinal Products*. London: European Medicines Agency.

Farinacci, M., Colitti, M., Sgorlon, V., and Stefanon, B. (2008). Immunomodulatory Activity of Plant Residues on Ovine Neutrophils. *Vet. Immunol. Immunopathol.* 126 (1-2), 54–63. doi:10.1016/j.vetimm.2008.06.006

Frédéric, M., Marcowycz, A., Cieckiewicz, E., Mégalizzi, V., Angenot, L., and Kiss, R. (2009). In Vitro anticanicidal Potential of Tree Extracts from the Walloon Region Forest. *Planta Med.* 75 (15), 1634–1637. doi:10.1055/s-0029-1185867

Fu, T., Elle, N., and Brunelle, A. (2018). Radial Distribution of Wood Extractives in European Larch Larix Decidua by TOF-SIMS Imaging. *Physicochemistry* 150, 31–39. doi:10.1016/j.phytochem.2018.02.017

Gao, Y., Zhou, L., Xiaoping, F., Chunyi, L., Jiayu, L., Lu, S., et al. (2016). Abietic Acid Attenuates Allergic Airway Inflammation in a Mouse Allergic Asthma Model. *Int. Immunopharmacol.* 38, 261–266. doi:10.1016/j.intimmp.2016.05.029

Garcia, G., Garcia, A., Gibernau, M., Bighelli, A., and Tosi, F. (2017). Chemical Compositions of Essential Oils of Five Introduced Conifers in Corsica. *Nat. Prod. Res.* 31 (14), 1697–1703. doi:10.1080/14712598.2017.1285299

Goad, L. J., and Goodwin, T. W. (1967). Studies on Phytosterol Biosynthesis: the Activity of Plant Residues on Ovine Neutrophils. *Vet. Immunol. Immunopathol.* 126 (1-2), 54–63. doi:10.1016/j.vetimm.2008.06.006

Holm, Y., and Hiltunen, R. (1997). Variation and Inheritance of Monoterpenes in Larix Species. *Flavour Fragr. J.* 11 (5), 234–239. doi:10.1002/(sici)1099-1842.2009.00048.x

Karlberg, A. T., and Lidén, C. (1985). Clinical Experience and Patch Testing Using Colophony. (IX). Sensitization Studies with Further Products Isolated from Larix Gmelinia (Rospr.). *Ruop. Wood. Phytother.* 10 (6), 478–482. doi:10.1002/doi:10.1002/0039-1567/199609104-478-aaid-pptr833-3.0.co;2-s

Kopania, E., Milczarek, S., Bloda, A., Wietecha, J., and Wawro, D. (2012). Extracting Galactoglucomannans (GGMs) from Polish Softwood Varieties. *Fibres Text. East Eur.* 20, 68 (96), 160–166.

Krüger, H. (1969). “Resina Laricis/Larix decidua”, in *Heilmittengaben Rudolf Steiner* (Dornach: Medizinische Sektion der Freien Hochschule für Geisteswissenschaften am Goetheanum).

Kubeckza, K.-H., and Schulze, W. (1987). Biology and Chemistry of Conifer Oils. *Flavour Fragr. J.* 2 (4), 137–148. doi:10.1002/doi:10.2730020402

Kuiters, A. T., and Sarink, H. M. (1986). Leaching of Phenolic Compounds from Leaf and Needle Litter of Several Deciduous and Coniferous Trees. *Soil Biol. Biochem.* 18 (5), 475–480. doi:10.1016/0038-0717(86)90003-9

Laireiter, C. M., Schnabel, T., Kock, A., Stalzer, P., Petutzschnig, A., Oostingh, G. J., et al. (2014). Active Anti-microbial Effects of Larch and Pine Wood on Four Bacterial Strains. *BioResources 9* (1), 273–281. doi:10.15376/biores.9.1.273-281

Lang, K. J. (1999). Die Zusammensetzung der Monoterpen-Fraktion in Zweigen von Larix decidua und L. kaempferi in Abhängigkeit von Jährseiten und Provenienz. *Phyton* 29 (1), 23–32.

Lang, K. J., and Messerer, M. (1987). Die quantitative Verteilung der Monoterpene in verschiedenen Teilen einer 19-jährigen Lärche (Larix decidua MILL.). *Phyton* 27 (2), 289–298.

Langenheim, J. H. (2003). *Plant Resins: Chemistry, Evolution, Ecology, and Ethnobotany*. Portland USA: Timber Press.

Lindner, W., and Grill, D. (1978). Säuren in Koniferennadeln. *Phyton* 18 (3–4), 137–144.

Malá, J., Cvikrová, M., Huřcová, M., and Máchová, P. (2013). Influence of Vegetation on Phenolic Acid Contents in Soil. *J. For. Sci.* 59 (No. 7), 288–294. doi:10.17221/23/2013-jfs

Matthews, S. M., Mills, L., Scalbert, A., and Donnelly, D. M. X. (1997). Extractable and Non-extractable Proanthocyanidins in Barks. *Phytochemistry* 45 (2), 405–410. doi:10.1016/S0031-9422(96)00873-4

Mecca, M., Todaro, L., and D’Auria, M. (2018). The Use of a Molybdenum Polyoxometalated Compound to Increase the Amount of Extractables from Wood Wastes. *Biomolecules* 8 (3), 62. doi:10.3390/biom8030062

Mills, J. S. (1973). Diterpenes of Larix Oleoressins. *Phytochemistry* 12 (10), 2407–2412. doi:10.1016/S0031-9422(73)80447-9

Mills, J. S., and White, R. (1987). “Natural Resins and Lacquers,” in *The Organic Chemistry of Museum Objects*. London: Butterworth & Co (Publishers) Ltd, 83–110. doi:10.17221/6987-0-408-11810-1.50013-1

Mofikoa, O. O., Mäkinen, M., and Jänis, J. (2020). Chemical Fingerprinting of Conifer Needle Essential Oils and Solvent Extracts by Ultrahigh-Resolution Fourier Transform Ion Cyclotron Resonance Mass Spectrometry. *ACS Omega* 5 (18), 10543–10552. doi:10.1021/acsomega.0c00991

Mulholland, D. A., Thuerig, B., Langat, M. K., Tamm, L., Nawrot, D. A., James, E. E., et al. (2017). Efficacy of Extracts from Eight Economically Important Forestry Species against Grapevine Downy Mildew (*Plasmopara Viticola*) and Identification of Active Constituents. *Crop Prot.* 102, 104–109. doi:10.1016/j.cropro.2017.08.018

Munn, Z., Peters, M. D. J., Stern, C., Tufanaru, C., McArthur, A., and Aromataris, E. (2018). Systematic Review or Scoping Review? Guidance for Authors when Choosing between a Systematic or Scoping Review Approach. *BMC Med. Res. Methodol.* 18 (1), 143. doi:10.1186/s12874-018-0611-x

Munteanu, I. G., and Apetrei, C. (2021). Analytical Methods Used in Determining Antioxidant Activity: A Review. *Ijmbs* 22 (7), 3380. doi:10.3390/ijmbs22073380
Willför, S., Sundberg, A., Hemming, J., and Holmbom, B. (2005). Polysaccharides in Some Industrially Important Softwood Species. *Wood Sci. Technol.* 39 (4), 245–257. doi:10.1007/s00226-004-0280-2

**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

**Publisher’s Note:** All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Copyright © 2022 Batista, Uecker, Holandino, Boylan, Maier, Huwyler and Baumgartner. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.