Biological activity of the birch leaf and bark

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Birch leaves and bark are traditionally used for folk medicine for centuries. Numerous research focused on the chemical composition and biological effects of birch extracts. The main pharmacologically active compounds found in extracts are flavonoids, saponins, tannins, essential oil, and triterpenes. Different species, age of the plant, geographical region, extraction methods, and analytical techniques influence the chemical composition. Possible role of birch extracts in preventing and treating numerous diseases is enabled by their diuretic, antimicrobial, anti-inflammatory, and anti-allergic activity. Therefore, this article is intended to give a general overview of the studies on birch leaves and bark.

Key words: biological activity; birch bark; birch leaf; chemical composition

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1. INTRODUCTION

The family Betulaceae, an important group of Angiosperms, which contains genera *Alnus*, *Betula*, *Carpinus*, *Corylus*, *Ostrya*, and *Ostryopsis*. There are numerous trees and shrubs of the genus *Betula* throughout the northern and western forested regions of Europe (Rastogi et al., 2015).

Since the beginning of healing practice, birch has been used in medicine. Leaves, bark and other parts of birch are often used in traditional and approved medicine (Shikov et al., 2021).

Birch bark, a significant byproduct of the agrochemical and wood-processing industries, is a source of numerous physiologically active compounds that could serve as the basis for the production of new, highly effective pharmaceutical agents (Abyshev et al., 2007).

Both birch leaf and bark represent a significant source of biologically active compounds. The traditional usage of birch leaves and bark in treating various diseases and conditions of the human body has initiated a large amount of pharmacological research (EMA, 2015; Tucakov, 1997). Due to the possibility of isolating a large number of pharmacologically active compounds, they present a significant source of raw materials for the cosmetic, pharmaceutical and food industries. This review aimed to systematize data on the chemistry and biological activity of birch leaves and bark so far.

2. METHODOLOGY

The literature was obtained from various multidisciplinary online accessible bibliographic databases, including Google Scholar , PubMed, SpringerLink, Scopus, and Web of Science.

3. PHYTOCHEMISTRY

Birch leaf contains 1-3.5 % flavonoid glycosides, among which hyperoside, quercetin, kaempferol, and myricetin glycosides are the most abundant (Ossipov et al., 1996). Respective flavonoid concentrations of 3.29 % and 2.77 % were measured in the dried leaves of *B. pendula* and *B. pubescens* (Lahtinen et al., 2006). Birch leaf (*Betulae folium*) is mentioned as the official drug in the 10th European Pharmacopoeia (Ph.Eur.10, 2019). It is made up of whole or fragmentary dried leaves of *B. pendula* Roth and/or *B. pubescens* Ehrh, as well as hybrids from both species, and it must contain a minimum of 1.5 % total flavonoids measured as hyperoside (Ph.Eur.10, 2019). Birch leaf also contains caffeic and chlorogenic acid, diarylheptanoids, lignans, triterpene alcohols, and malonyl esters damarene-type (Hänsel and Sticher, 2010).

The highest phenol content was found in early May, with a levels dropping in mid-July, and an increase in late summer and autumn, according to the research on the seasonal oscillations of phenols in the leaves of *Betula* spp. (Kavelenova et al., 2001). Birch leaves synthesize polymer proanthocyanidins. The amount of these compounds in plant species *B. pendula* was 39 mg/g (Karonen et al., 2006). The lipid and fatty acid composition of *B. pubescens* and *B. pendula* leaves varies. Yellow leaves have high concentrations of saturated fatty acids, glycolipids, and linolenic acid (Shulyakovskaya et al., 2004). It was discovered that the birch leaf contained potassium. The potassium concentration in the *B. pendula* leaf was 8045 μg/g of dry material, while the sodium concentration was 40 μg/g (Szentmihályi et al., 1998). Additionally, the leaves contain tannins, sterols, coumarins, carotenes and vitamins (2-8 %, including ascorbic acid with about 0.5 %, nicotinic acid, etc) (Turova et al., 1987).

The birch leaves have an essential oil content of 0.05-0.1 %, mostly made up of sesquiterpene oxides (Szentmihályi et al., 1998).

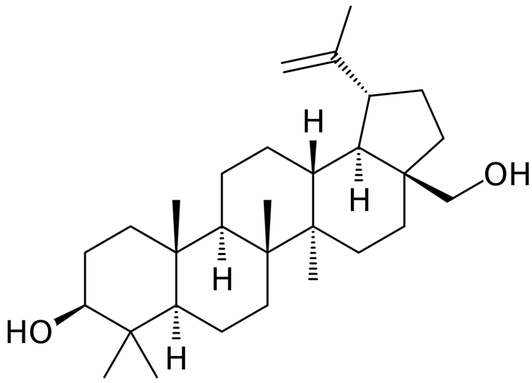

Fig. 1. Chemical structure of betulin

The outer layer as well as the inner layer make up the two layers of birch bark.The outer layer of the bark contains the most extractive compounds. Betulin (Figure 1), a pentacyclic triterpenoid, is the primary ingredient in almost all extracts of the bark, and it gives the bark its characteristic white appearance (Kuznetsova et al., 2014). Betulin can make up to 20–30 % or even 45 % of the bark's dry mass, depending on the geographical origin and the biological source (birch species). Inconsistent calibration procedures and various analytical methods can also cause variances (Demets et al., 2022). Birch bark may contain betulone, erythrodiol, and oleanolic acid in much lower concentrations (Jäger et al., 2008). In comparison to the betulin content in the outer bark, the European species *B. pendula* Roth. may contain 10–20 % betulinic acid and lupeol (Figures 2 and 3). It should be mentioned that lupeol and betulinic acid are the main contaminants of raw betulin (Orsini et al., 2015).

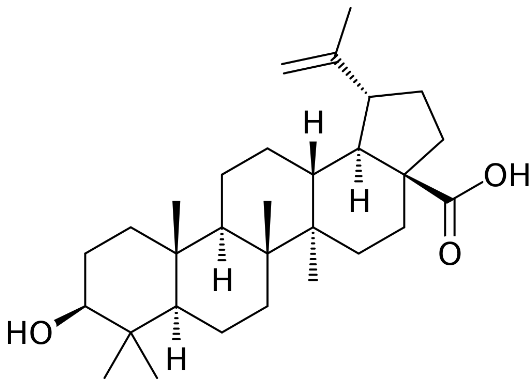

Fig. 2. Chemical structure of betulinic acid

Betulin posess numerous pharmacological effects such as antibacterial, antiviral, antitumor, etc. Because of its pharmacological properties, betulin has the potential to be used in a number of industries, including the pharmaceutical and medical ones. Betulin is a valuable natural source that can be used in both its unprocessed and transformed forms due to its wide range of biological activity and availability (Demets et al., 2022).
The average chemical content of *B. pendula*, *B. papyrifera*, and *B. neoalaskana* extracts is shown in Table 1 (Krasutsky, 2006).

B. pendula and *B. pubescens* bark's chemical composition has been thoroughly analyzed in a number of research (Abyshev et al., 2007). These studies have shown that different groups of chemical compounds, including essential oils, carbohydrates, carotenoids, coumarins, flavonoids, hydrocarbons, saponins, tannins, terpenoids, etc., are present in bark extract. A group of pentacyclic triterpenoids, lupane, is the main component of birch bark among other chemical compounds present. Lupeol, lupeol-3-acetate, betulinol, betulinic acid, betulinol aldehyde, etc. are some of the pentacyclic triterpene compounds present in birch bark (Abyshev et al., 2007).

4. TRADITIONAL USE

Birch leaves have been used since ancient Greek and Roman times. Teutonic tribes used birch leaves in potions to increase strength and beauty. In traditional medicine, birch leaf is used to purify the blood and as a pain reliever for gout, arthritis, rheumatism, and other types of pain. Hair loss and dandruff are prevented with the use of products containing the leaf of birch (PDR, 2000). Birch is a popular remedy in traditional Russian medicine used to cure a variety of disorders (Shikov et al., 2021). Gout and articular rheumatism can be treated with birch leaf baths. The steamed leaves are applied topically to reduce neuralgic pain and treat rheumatism (Shikov et al., 2021).
Herbal medicines are also widely used in Eastern and Southeastern Serbia. Birch leaves, bark, and seeds are used in folk medicine to treat various skin conditions, productive cough, common cold, and renal colic in the Svrljig and Timok regions, while they have also been used to treat prostate disease and headache in the Svrljig region (Matejić et al., 2020; Tucakov, 1997).
The German Commission E recommends birch leaf as a diuretic and states its use in treating urinary tract inflammation and infections, kidney gravel, as well as its use as supportive therapy for rheumatic diseases (Blumenthal et al., 1998). The European Medicines Agency's committee on herbal medical products mentions the traditional use of comminuted and powdered herbal material, dry extract, and liquid extract as a diuretic and adjuvant in the therapy of uncomplicated urinary system diseases (EMA, 2015).The comminuted herbal material (2-3 g) in 150 mL boiling water is used for the preparation of herbal tea and is consumed up to 4 times a day (EMA, 2015).
European Union herbal monograph on Species diureticae indicates the evidence of traditional medicinal use of birch leaves in a range of 10–57 % in policomponent tea combination or "traditional herbal medicinal product to increase the amount of urine to achieve flushing of the urinary tract as an adjuvant in minor urinary complaints" (EMA, 2017). The ESCOP monograph of *Betulae folium* approves its use, primarily in cases of inflammation and kidney stones, as well as an adjuvant in the therapy of bacterial infections of the urinary tract (ESCOP, 2009).
In addition to the many uses of birch leaf, birch bark is becoming increasingly popular. Even though EMA still has not published an herbal monograph, numerous studies that support the use of birch bark for certain indications have been published.

5. BIOLOGICAL ACTIVITY

5.1. Effect on the urinary system/diuretic activity

The decoction, infusion, powder, water-ethanol, butanol extract, and birch leaf extract obtained with carbon dioxide, as well as individual isolated substance, were tested on several animal models to evaluate their diuretic effect (EMA, 2017). Vollmer and Hübner (1937) investigated the effect on diuresis

Table 1. The average chemical composition of birch bark extracts (Krasutsky, 2006)

Chemical composition	*Betula pendula* [%]	*Betula papyrifera* [%]	*Betula neoalaskana* [%]
Betulin	78.1	72.4	38.1
Betulinic acid	4.3	5.4	12.5
Betulinic aldehyde	1.2	1.3	1.4
Lupeol	7.9	5.9	2.1
Oleanolic acid	2	0.3	2.2
Oleanolic acid 3-acetate	-	1.6	3.8
Betulin 3-caffeate	0.5	6.2	6.1
Erithrodiol	2.8	-	-
Other	3.2	6.9	3.8

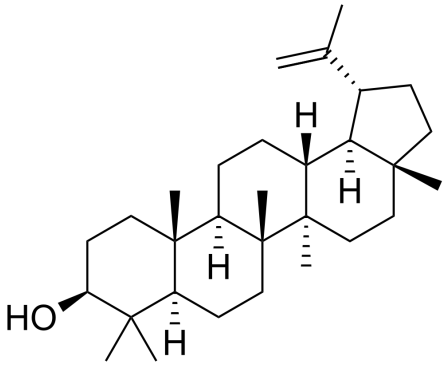

Fig. 3. Chemical structure of lupeol

of different doses of birch leaf infusion on rabbits and mice. The test results showed that, depending on the dose of birch leaf infusion, urine volume and chlorine excretion in rabbits increased by about 19 % and 41 %, while urine volume in mice increased up to 42 %, and chlorine excretion went up to 128 %. Administration of birch leaf infusion in rats did not lead to an increase in urine volume, but rather an increased elimination of urea and chlorine (Vollmer and Hübner, 1937).

Borkowski (1960) researched the effects of birch leaves on dogs. He noted that urine volume was increased by 13.8 % 2 hours after oral administration of birch leaf powder. More research on rats showed that oral administration of aqueous and alcoholic extracts increased urine excretion. The aqueous extract produced the best results. After 3 hours, a dose of 5.32 mL/kg body weight increased urine output by about 54 %. Sodium, potassium, and chlorine excretion were insignificant (Schilcher et al., 1989). The effects of the ethanolic birch leaf extract were also examined: the extraction solvent 70 % ethanol was fractionated with butanol and water. No change in urine and ion excretion was detected in rats after oral administration of an ethanolic, butanol, and aqueous extract. No diuretic effect was observed after oral administration of dammaran-type esters to male Wistar rats (Rickling and Glombitza, 1993). Two hours after the intragastric administration of one dose of 10 mg/kg extract of *B. pendula* leaves in rats, urine output increased 1.3-fold in comparison to the control (Chumak et al., 2019).

Also, birch leaf is used to treat urinary tract infections. A randomized, double-blind pilot study showed that patients who consumed birch leaf tea had a reduction in the number of microbes in their urine compared to a placebo group (Engesser et al., 1998). In the study by Müller and Schneider (1999), 1066 patients were included, who were classified into four groups. The first group of patients suffered from urinary tract infections; the second group suffered from kidney stones; the third group suffered from the irritable bladder, and the fourth group suffered from other health issues. Every patient got different daily doses of a dry aqueous birch leaf extract (from 180 to 1080 mg or more). In addition to the aqueous birch leaf extract, a part of the first group also got antimicrobial treatment. In 80 % of the treated patients, the symptoms disappeared after 2–4 weeks (Müller and Schneider, 1999).

5.2. Antimicrobial activity

Research on the aqueous extract of birch leaves *in vitro* led to observing its virostatic and cytostatic properties (EMA, 2017). A carbon dioxide birch leaf extract showed antibacterial activity against *Staphylococcus aureus*. Birch leaf extract prepared with 70 % acetone also showed antibacterial activity against this bacterial strain (Rauha et al., 2000).

The 400 $\mu g/cm^3$ concentration of birch leaf essential oil demonstrated a significant antimycotic activity when tested on a variety of fungi, essential oils have been found to fully stop the growth of *Rhizoctonia cerealis*, and *Cephalosporium aphidicola*. Depending on the strain under study, different inhibitory effects on fungal growth were observed (Demirci et al., 2000). Methanol dry extract of *B. pendula* leaves had an inhibition zone of 12 mm (MIC 2.5 %) against *Staphylococcus aureus* and 11 mm against *Pseudomonas aeruginosa*, while aqueous decoction (1:10) had an inhibition zone of 13.4 mm against *Staphylococcus aureus* (Đurić et al., 2013).

Pre-clinical research pointed out the significant antimicrobial effect of birch bark extract against *Mycobacterium tuberculosis* and pronounced positive effects on the reparative processes in the organs affected by the infection. *Mycobacterium tuberculosis* is an intracellular bacteria that makes it difficult to fight infection. Currently available drugs are used in combination. Unfortunately, cases of intolerance, as well as adverse reactions to the administered drugs often occur. The effect of dry birch bark extract on the *Mycobacterium tuberculosis* infections in mice was investigated. The ability of birch bark extract to reduce the growth of *M. tuberculosis* both *in vitro* and *in vivo* was noted, and the lungs, liver, and spleen of the injured mice also showed signs of improvement in their healing processes. Also, high concentrations of the medicine led to a decrease in macrophage lysis. The results of testing the antimycobacterial activity of birch bark testify to the justification of clinical trials in patients with tuberculosis (Demikhova et al., 2006). In a clinical study involving 60 patients (aged 20 to 55) with newly discovered destructive tuberculosis, the patients were split up into various groups. Twenty patients from the first group got the standard treatment (isoniazid, rifampicin, pyrazinamide, and ethambutol) in combination with 2 capsules of medicine containing 100 mg of birch bark extract 2 times a day over 3 months. The second group received 4 capsules 2 times a day during the same period. Patients from the third group received only the standard treatment (isoniazid, rifampicin, pyrazinamide, and ethambutol) for 3 months. Comparative analysis of the dynamics of intoxication, clinical signs, and normalization of clinical blood analysis in patients from the first and the second group showed beneficial impact of birch bark extract on the progression of the disease in combination with the standard treatment. The combined treatment made it possible to stop the intoxication syndrome 2 times more effective in the first month of the treatment. In 90-95 % of patients who used the birch medicine, the intoxication disappeared already by the beginning of the second month. Based on clinical and laboratory data, a positive influence of birch bark on pulmonary tuberculosis was determined (Demikhova et al., 2006). Numerous research into the antibacterial properties of birch bark have been done. The results indicated that birch bark shows antibacterial activity due to its active principles: betulinic acid and its derivatives (Rastogi et al., 2015). High antibacterial abilities were shown by birch bark extracts against Gram-positive bacteria such as MRSA, *Staphylococcus epidermidis*, and *Cutibacterium acnes* (Emrich et al., 2022). Studies with a variety of Gram-negative bacteria, such as *Pseudomonas aeruginosa*, *Salmonella typhi*, and *Klebsiella pneumoniae*, revealed that they did not respond as well to bark extract therapy as Gram-positive species did. A study on the antibacterial properties of bark and wood extracts found that a greater variety of wood and bark extracts can inhibit a wider range of Gram-positive bacteria than Gram-negative bacteria (Vainio-Kaila et al., 2017).

The antiviral activity of birch bark extract towards Herpes simplex virus type 1 was investigated *in vitro* (Aiken and Chen, 2005). Birch extract had a high level of activity toward viruses when it was administered before infection. Pentacyclic triterpenes prevented either acyclovir-sensitive or acyclovir-resistant strains of HPV-1 at an initial phase of the disease. The evaluation of the *in vivo* antiherpetic effect of extract from birch bark requires clinical trials (Heidary Navid et al., 2014). Birch bark, more precisely betulinic acid, as well as its derivatives, demonstrated to prevent the HIV-1 virus from replicating in the early stages of its development. Thus, they also have the potential for anti-HIV therapy. Betulinic acid and its derivatives are an example of non-peptide compounds that have the potential to inhibit HIV-1 virus replication (Shikov et al., 2011).

5.3. Antimutagenic and antitumor activity

In an *in vivo* study on mice, an aqueous decoction of *B. pendula* leaves reduced pulmonary metastasis and decreased tumor cell proliferation (Molokovskii and Dyachuk, 2006).

Cisplatin is one of the most potent clinically effective compounds with anticancer properties that are still in use copper, magnesium, iron, manganese, and zinc are some of the elements that become unbalanced after being exposed to cisplatin. When birch sap was applied following the administration of cisplatin, zinc, magnesium, copper, iron, and manganese levels were either normalized or raised, indicating that the treatment with birch sap restored the organism's natural homeostatic condition (Muselin et al., 2018).

Birch bark has become the subject of numerous studies due to its high content of betulinic acid, which selectively inhibits tumor growth and induces cell apoptosis. Animal testing has showed that betulinic acid inhibits the development of melanoma without damaging healthy cells (Wang et al., 2017). In colorectal carcinoma cells, betulinic acid has the ability to suppress the phosphorylation autophagy regulating factor (Wang et al., 2020). Using ubiquitin arrays, the scientists discovered that betulinic acid can decrease the amount of ubiquitination in several proteins, inhibiting the expression of hypoxia-inducible target genes (Zheng et al., 2019). In animal xenograft tumor models, betulinic acid increased glucose-regulated protein 78 levels and decreased matrix metalloproteinase levels in breast cancer metastatic lesions in the lungs, indicating its ability to reduce breast cancer invasion and metastasis *in vivo* (Liu et al., 2012). Recent research has also revealed that betulinic acid may interact with or bind directly to target molecules such as glucose-regulated proteins and cannabinoid receptors, thus mediating anticancer effects (Cai et al., 2018). Also, betulinic acid activates the mitochondrial pathway of cell apoptosis by increasing the production of reactive oxygen species but without the involvement of the p53 protein system or CD95 receptor/ligand. It suppresses topoisomerases I and IIa, as well as aminopeptidase N, and it prevents angiogenesis (Hordyjewska et al., 2019). In addition to betulinic acid, birch bark contains betulin, which can cause a transient reduction in DNA replication by affecting the cycle-regulating genes, p21 and p53 (Otto and Sicinski, 2017). Birch bark is a significant source of physiologically active compounds with anticancer action, according to previous research. One of the active ingredients is a class of chemicals known as pentacyclic triterpenes. It is thought that these compounds work in concert with other substances found in bark to produce a more potent effect.

By using methanol and methylene chloride for extraction, the common birch extract was created. Both extracts showed significant anticancer properties. Despite having weaker activity than a methylene chloride extract, a methanol extract has an advantage over it because it does not cause cell necrosis (Goun et al., 2002).

Three human cell lines, A431 (skin epidermal carcinoma), MCF7 (breast adenocarcinoma), and HeLa (cervical adenocarcinoma) were used to investigate the antiproliferative activities of a common birch bark extract, betulin, and betulinic acid standards. Chloroform, dichloromethane, and methanol were used to prepare birch bark extract by the Soxhlet method. Birch bark extract was found to have strong antiproliferative properties similar to those of betulin and betulinic acid (Rastogi et al., 2015). To investigate the effect of cell proliferation suppression, an extract of birch bark containing 90 % betulin was tested *in vitro* on malignant tumor cell lines. All of the prepared extracts demonstrated a substantial inhibitory effect on tumor cell proliferation (Rastogi et al., 2015).

Betulin is still not frequently used, but it is thought that it will play a significant role as a natural substance and as a precursor to the creation of other physiologically active molecules in the future. When betulin is transformed into betulinic acid, several different biochemical and physiological effects are observed. Because they have effects on a number of malignancies, betulin, betulinic acid, and its derivatives may have therapeutic use (Lombrea et al., 2021).

The biosphere is becoming more and more polluted on a global scale, which is increasing the mutagen load. The identification and removal of genotoxicants from the human environment is the most efficient form of preventive action against induced mutagenesis. However, for both financial and medical reasons, it is impossible to rule out many mutagenic substances. Around the world, research is being done to find chemicals that can lower the degree of mutagenesis and increase resistance to mutagenic impacts. To study the impact of birch bark extract on dioxidine and cyclophosphamide-induced spontaneous and induced mutagenesis in mice 50, 150, 450, and 1500 mg/kg of dry birch bark extract were given orally, while dioxidin and cyclophosphamide were given intraperitoneally. Three different versions of the experiment were run. In the first version, the mutagen and dry birch bark extract were both administered once. In another variant, dry birch bark extract was administered daily for five days. In the third version, the mutagen and birch bark extract were given simultaneously for five days. Following that, C57Bl/6 mouse cells with chromosomal damage were recorded. According to this study, birch bark extract has a protective effect (about 60 %) that is comparable to the pharmaceutically active derivatives of 2-mercaptobenzimidazole, the most potent antimutagenic compound (Zhanataev et al., 2004). However, birch bark extract has some undeniable advantages: natural origin and absence of side effects. Confirmation of efficacy in clinical trials will open wide possibilities for its multifunctional application (as a drug, bioactive substance, or food additive) for the protection of the human genome (Abyshev et al., 2007).

5.4. Anti-inflammatory and antiallergic activity

Today, different antihistamines are common as a treatment for allergies. However, the search for new effective antiallergens is ongoing, especially since the spectrum of allergens is further increasing due to the successive deterioration of the global ecological situation. Finding new antiallergens of natural, herbal origin, which can suppress the activity of phospholipase A2 and minimize side effects during application is of particular importance. Long-term use of corticosteroid antiallergic agents reduces the resistance to infections, disrupts metabolism, especially metabolism of carbohydrates, lipids, and proteins, suppresses the activity of the hypothalamic-pituitary-adrenal axis, causes liver damage, nervous disorders, and many other side effects. Birch bark extract containing betulin has an anti-allergic effect comparable to drugs such as chloropyramine and loratadine. Birch bark extract was found to have an effect similar to chloropyramine in the treatment of mild and severe anaphylactic shock, and to decrese the systemic allergic reaction in 50 % of the tested guinea pigs inoculated with egg white ovalbumin. The amount of IgE antibodies to ovalbumin in mice is significantly reduced when birch bark extract is administered orally or intravenously. A direct impact of birch bark extract on H1 receptors was not observed (Kovalenko et al., 2007).

The ability of birch bark, more specifically its components betulinic acid and betulin, to trigger and modify cytokine production in human entire blood cell cultures was researched (Kovalenko et al., 2007). The results of the trial showed that betulin can cause a low but detectable increase in TNF-α levels in humans. However, it is ineffective in inducing other pro-inflammatory cytokines such as IFN-γ and anti-inflammatory IL-10. Unlike betulin, betulinic acid was ineffective in inducing cytokines (Kovalenko et al., 2007).

In *in vitro* research, lyophilized aqueous extract from *B. pendula* leaves demonstrated mild prostaglandin synthesis inhibition and medium suppression of platelet activating factor-induced exocytosis (Shikov et al., 2021). An aqueous dry extract of *B. pendula* leaves considerably lowers ocular inflammation after keratoplasty in Lewish rats (Wacker et al., 2012). The growth and cell division of $CD8^{+}$ and $CD4^{+}$ activated T lymphocytes but not resting T lymphocytes was significantly and dose-dependently inhibited by *B. pendula* leaf aqueous dry extract (Gründemann et al., 2011).

5.5. Hepatoprotective effect

A study involving patients who had chronic hepatitis C was done to examine the potential of birch bark abilities to heal the liver (Zdzisiñska et al., 2003). Forty-two patients with diagnosed chronic hepatitis C participated in the study and received daily treatment 160 mg of standardized birch bark extract for twelve weeks. The parameters for the primary and secondary outcomes were evaluated. Alanine aminotransferase has been the primary outcome parameter measured after twelve weeks. Additional measurements included the aspartate aminotransferase profile, Hepatitis C virus RNA values, and subjective symptoms and signs of the illness. To validate the presence of 75 % betulin and 3.5 % betulinic acid, a qualitative-quantitative analysis of birch bark extract was carried out using high-performance liquid chromatography. After twelve weeks of treatment, evident variations in average Hepatitis C virus RNA values and alanine aminotransferase were detected. Hepatitis C virus RNA was decreased by 43.2 %, while the amount of alanin aminotransferase was decreased by 54.0 % and normalized. After receiving treatment for twelve weeks, reports of weariness and stomach discomfort decreased by sixfold and threefold, respectively, and dyspepsia was no longer observed. Future controlled clinical trials will be necessary to establish the data's clinical application because a control group wasn't included in this inquiry (Zdzisiñska et al., 2003).

Birch bark extract was discovered to have high hepatoprotective and detoxifying effects, normalize hemopoiesis lower triglyceride levels, and possess hypolipidemic qualities, making it appropriate for use in therapy of liver illnesses (Hordyjewska et al., 2019).

5.6. Other activity

After six days of intragastric treatment, an aqueous *B. pendula* leaf decoction in mice lowered liver glycogen levels and decreased the amount of malondialdehyde in the gastric mucosa (Molokovskii and Dyachuk, 2006).

An open, randomized, multicenter study investigated the intraindividual effectiveness and acceptability of a gel for treating burns that contains birch bark extract. Acute superficial burns in 57 patients were randomly treated with a gel containing birch bark extract or Octenilin® wound gel. In comparison to Octenilin® wound gel, the percentage of patients with faster tissue regeneration was much wider for the gel containing birch bark extract (Frew et al., 2019). Also, the aim of a randomized clinical trial conducted by Barret et al. (2017) was to analyze how the topical gel Episalvan that contains 90 % sunflower oil and 10 % birch bark extract helps to accelerate epithelialization. Wounds closed faster in patients treated with gel than without it (Barret et al., 2017).

In the Czech Republic and other countries in Central and Eastern Europe, researchers have looked at the *in vitro* inhibitory effects of botanicals that have traditionally been used to treat gout, arthritis, and rheumatism (Havlik et al., 2010). The study involved 80 % ethanolic and methylene chloride-methanolic extracts of birch leaves. According to this study, birch is effective in treating gout and rheumatoid arthritis (Havlik et al., 2010).

5.7. Other toxicity

So far, there are no known compounds that can be isolated from birch leaves and/or bark that could pose an increased risk. Birch leaf extract showed weak mutagenic effects attributable to quercetin. *In vitro* genotoxicity experiments on quercetin are known to produce positive outcomes. *In vivo* effects, however, have not been observed. The genotoxicity of birch leaf extract was investigated. During the AMES assay, birch leaf extract had a weak mutagenic effect on the *S. typhimurium* strains TA 98 and TA 100 (Göggelmann and Schimmer, 1986). A weak mutagenic effect is attributed to quercetin. Most studies have shown no carcinogenicity for quercetin. Reproductive toxicity studies were not performed. Birch bark extract belongs to the fifth class of toxicity (food toxicity). Research conducted at the RAMS Institute of Pharmacology has shown that birch bark extract has no toxic, allergic, mutagenic, or co-mutagenic effects, as well as no teratogenic and embryotoxic effects. The acute toxicity test of dry birch bark extract was conducted on non-linear male mice with a body weight of 18-20 g. The preparation was administered intragastrically in doses up to 17 g/kg. During the entire research period, no animal deaths occurred. This fact provides the basis for the conclusion that the LD_{50} of the dry extract of birch bark is greater than 17.0 g/kg and that it can be classified as a substance of low toxicity (Kuznetsova et al., 2014). Jäger et al. (2008) investigated the subchronic toxicity of a triterpene extract of birch bark after intraperitoneal and subcutaneous application to dogs. A subchronic toxicity study's findings show the birch bark triterpene extract is safe for using (Jäger et al., 2008). Because of a lack of safety information, birch leaf use is not recommended in those who are pregnant, breastfeeding or younger than twelve years old (EMA, 2017).

CONCLUSION

Birch has been used in medicine for a very long time. It has been verified that using birch leaves as a traditional herbal remedy can influence the volume of urine excreted and help with minor urinary issues. There are insufficient clinical studies in patients with urinary tract infections to support a well-established use in this indication. Due to the absence of toxic ingredients, the lack of reports of severe adverse effects, and their long use, the safety of traditional herbal remedies can be assumed to be acceptable. Clinical studies should be conducted on special populations, such as the elderly and children.

Birch bark extract is characterized by non-toxicity and, as a consequence, it could be considered safe for use in both humans and animals. Numerous studies have been carried out to investigate its effects. Birch bark shows a high level of adaptogenic, antioxidant, hepatoprotective, detoxifying, hypolipidemic, immunomodulatory, anti-inflammatory, anti-allergic, and antiviral activity.

Due to the pharmacologically active components, birch leaf, and bark represent a very important resource to be used in future formulations of a large number of natural medications with a wide range of applications.

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