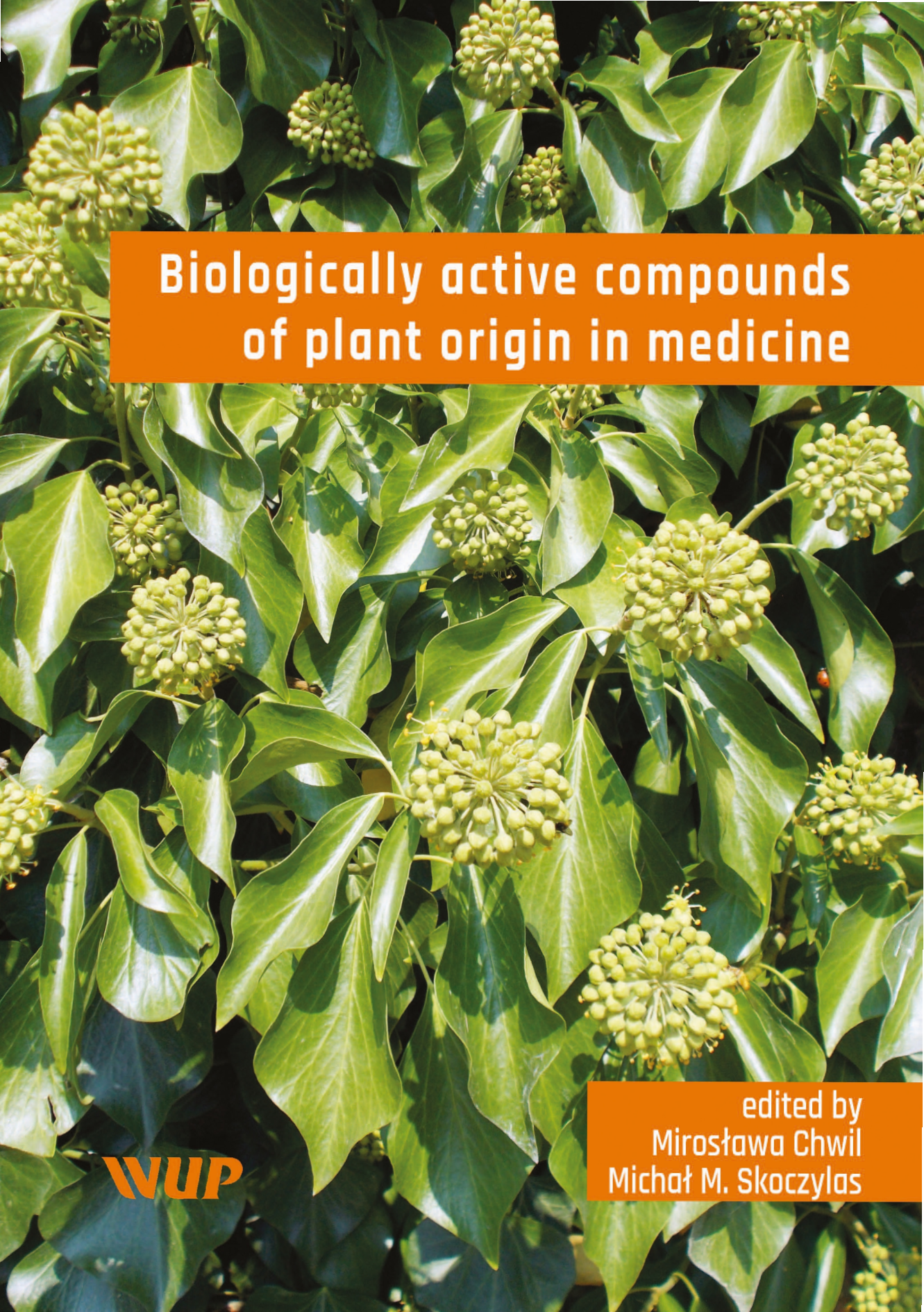

Biologically active compounds of plant origin in medicine

edited by
Mirosława Chwil
Michał M. Skoczylas

WUP

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Wydawnictwo Uniwersytetu Przyrodniczego w Lublinie

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Lublin 2020

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Contents

Foreword

The monograph *Biologically active compounds of plant origin in medicine* provides the Reader with information about biologically active substances with focus on the scientific background of their use in medicine and nutrition. At the beginning of the third decade of the 21st century, it is possible to investigate the beneficial effects, therapeutic application, and safety of many bioactive compounds contained in herbal blends and food. To highlight this potential, we recapitulate the results of modern studies on the importance and use of phytocompounds in medicine. The contemporary findings confirm the achievements of ancient and folk medicine, discover novel effects of well-known compounds, and identify new natural substances with elucidation of their mechanisms of action. We hope that these reviews will provide the Reader with knowledge of the current issues in this field.

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Grażyna ZAWIŚLAK[1]

Plant development phase as a determinant of the composition and quality of essential oil in selected plants of the family Lamiaceae

Summary. Essential oils are the basic active compounds in plants of the family Lamiaceae. The content of oils in the plant varies during the vegetation season. The qualitative and quantitative composition of essential oils is modified by plant ontogenesis. Knowledge of the trend in the changes in the chemical composition of essential oils can help to identify a harvest term in which the oil exhibits the highest accumulation of compounds determining its strong aroma and therapeutic properties. The basil, hyssop, lemon balm, and savoury essential oils described in the paper are commonly known. In contrast, the horehound oil is a rare substance.

Key words: basil, hyssop, lemon balm, savory, horehound

Introduction

Essential oils are biologically active compounds present in many herbal raw materials. They are poisonous to plants but valuable for the human organism due to their broad spectrum of therapeutic effects. With their specific aroma, essential oils are regarded as valuable spices and flavouring additives in food production. Each essential oil-bearing plant has a specific scent associated with the volatile nature of the oils. Many researchers indicate that the smell of aromatic herbs can be modified by ontogenetic and environmental factors [Kudełka and Kosowska 2008, Janas et al. 2012, Nurzyńska-Wierdak et al. 2012, Bazaid et al. 2013]. This is associated with changes in the qualitative and quantitative composition of essential oils during the vegetation season [Kicel et al. 2005, Mirjalili et al. 2006, Kudełka and Kosowska 2008, Janas et al. 2012, Verma et al. 2012, Toncer et al. 2017].

The Lamiaceae family comprising approximately 236 genera and over 6000 plant species is a distinguished representative of the enormous biodiversity of herbal oil plants [Naghibi et al. 2005, Koocheki et al. 2008, Satil et al. 2008,

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Grozeva and Budakov 2010, Honermeier et al. 2013, Mamadalieva et al. 2017]. Lamiaceae plants exhibit an ability to synthesise essential oils, but their health-promoting value is also associated with the presence of other substances with antioxidant, anti-inflammatory, antimicrobial, immunomodulatory, and anti-cancer activity [Nassar et al. 2015, Rai et al. 2016, De Assis et al. 2018].

Ocimum basilicum L.

The oil substantially increases the antioxidant potential of basil raw material [Nurzyńska-Wierdak and Zawiślak 2016]. The content of essential oil in the common basil herb may range from 0.5 to 2.5% [Nurzyńska-Wierdak 2012].

As reported by Golcz et al. [2006], the oil content varied depending on the plant development phase and ranged from 0.34% (at the beginning of plant flowering) to 0.45% (in the full flowering phase). It was shown by Nurzyńska-Wierdak et al. [2012] that the content of essential oils in the herb of two basil varieties was slightly higher (0.83% in 'Kasia' and 0.75% in 'Wala') and increased along the plant development phases. It should be emphasised that both varieties had high content of essential oil with a composition changing during ontogenesis. The oil extracted at the flower bud stage was characterised by the highest content of linalool (in both varieties) and 1,8-cineol (only in 'Kasia'). Plant development was accompanied by a decline in the concentration of methylchavicol and methyl eugenol. Methylchavicol and methyl eugenol are regarded as mutagenic compounds, which limits the use of basil oil considerably. The ontogenetic variability is particularly important, as it largely determines the appropriate basil harvest term as well as the chemical composition and therapeutic properties of basil essential oil. As reported by Toncer et al. [2017], basil oil extracted from plants before flowering was rich in eugenol. In turn, it was dominated by linalool during the flowering period, whereas terpinolene-4-ol was found to be the main compound after the flowering phase. It was demonstrated that the composition of essential oil in basil growing in Oman was modified by the season of year. β-farnesene was detected as one of the main components of basil essential oil in winter and spring, whereas in summer it was present only in trace amounts [Al-Maskria et al. 2011].

Hyssopus officinalis L.

The plant development phase was shown to modify the content of essential oil in hyssop herb. It gradually increased until the full flowering phase (1.65%), which was followed by a decrease in its concentration in the plant [Kizil et al. 2008, Zawiślak 2011b, 2013b]. A similar relationship was reported by Kizil et al. [2016], who analysed changes in the content and composition of essential

oil in hyssop plants cultivated in a semi-arid area in Turkey. In turn, as shown by Kara and Baydar [2012], the level of essential oil in hyssop plants during the full flowering phase decreased.

Various analyses of qualitative and quantitative changes in hyssop oil have demonstrated an important role of ontogenetic factors. Studies conducted by Khazil et al. [2008, 2010, 2016] showed that *trans*-pinocamphone was the dominant component of hyssop oil (47.9–51.4%, 57.2%, 47.2–72.1%), regardless of the plant development phase. The highest level of *trans*-pinocamphone in the full flowering phase was also reported by Moghtader [2014]. Different findings were reported by Zawiślak [2013b], i.e. the highest concentration of *trans*--pinocamphone was detected in oil from plants harvested in the vegetative phase (43.5%); however, its content decreased during plant development and reached the level of 15.5% in the full flowering phase. Concurrently, the *cis*--pinocamphone content increased and reached a maximum concentration of 54.9% in fully flowering plants. The highest level of *cis*-pinocamphone in hyssop oil was also reported by Németh-Zámbori et al. [2017] and Baj et al. [2018].

The change in β-pinene levels reported in a study conducted by Zawiślak [2013b] was similar to that in the content of *trans*-pinocamphone. The highest concentration of this ingredient was found in essential oil obtained from plants harvested in the vegetative phase, whereas a twofold lower content was determined in oil extracted from plants collected at the beginning and full flowering. The content of β-pinene in hyssop oil does not always change substantially during the growing season [Kizil et al. 2016].

Kara and Baydar [2012] reported dominance of sabinene in hyssop oil in all plant development phases. In contrast, sabinene was not indicated as a dominant compound in hyssop oil in other studies [Kizil et al. 2008, Zawiślak 2013a, 2013b, Moghtader 2014, Kizil et al. 2016, Zawiślak 2016, Baj et al. 2018].

In practice, collection of essential oil in the initial flowering phase is usually recommended. It offers plants a possibility of producing new shoots, which are the raw material to be collected in a subsequent harvest. Hence, herb can be repeatedly harvested during one vegetation season. Zawiślak [2016] assessed the chemical composition of essential oil derived from hyssop material collected at the onset of flowering (beginning of July) and from newly produced shoots (end of August) and detected stable levels of camphene, elemol, germacrene D, bicyclogermacrene, and dodecane. The author also demonstrated changes in the content of other dominant oil compounds. The content of β-pinene, i.e. an ingredient used in cosmetics and food production, was the largest at the beginning of hyssop flowering (10.8%) but declined to 7.0% at the end of August. It is therefore important to identify the most favourable term for harvesting hyssop herb (the initial flowering phase), especially when its raw material is intended to be used as a natural source of aroma.

Melissa officinalis L.

Lemon balm is a plant with low content of essential oil (0,13–0,35%); nevertheless, it emits a specific lemon scent [Pouyanfar et al. 2018]. The content and the qualitative and quantitative composition of its oil are significantly influenced by the plant development phase [Khizil 2009, Saeb and Gholamrezaee 2012, Nurzyńska-Wierdak 2013, Uyanik and Gurbuz 2015]. Analysis of changes in the content of compounds in lemon balm oil helps to indicate an appropriate harvesting term to ensure the highest quality of the raw material. Geraniol is one of the dominant ingredients in lemon balm oil [Sharafzadeh et al. 2011, Verma et al. 2015, Seidler-Łożykowska et al. 2017, Pouyanfar et al. 2018]. Large fluctuations in the content of this compound in the essential oil of the plant were detected throughout the plant ontogenesis period. Before and during flowering, the content of geraniol reached 25.03% and 24.97%, respectively. After flowering, its levels declined to 4.58%. A similar downward trend was noted for caryophyllenne oxide. The content of the compound in oil from plants harvested before flowering accounted for 8.75%. A slight decrease in its content was recorded in the flowering phase (7.55%), whereas a level of only 0.95% was detected in the lemon balm after the flowering phase. Considering the plant development phases, a several-fold increase in the level of some compounds has been demonstrated at the end of the lemon balm vegetation season. The carvacrol content of ca. 2% before and during the flowering phase was found to increase to 37.62% after flowering. Equally noteworthy is the significant increase to 32.34% in the content of methyl citronellate in oil extracted from lemon balm plants after the flowering period. Before and during the flowering phase, the level of methyl citronellate in the lemon balm oil was low, i.e. 0.98% and 0.33%, respectively [Saeb and Gholamrezaee 2012]. As reported by Uyanik and Gurbuz [2015], caryophyllenne oxide was indicated as the main component of lemon balm oil at all development phases, except for the pre-flowering phase, in which the oil contained the highest content of citral, followed by caryophyllenne oxide.

Satureja sp.

The genus *Satureja* comprises plants with a highly diverse composition of essential oil. Raw materials are collected both from cultivations and from natural habitats [Nurzyńska-Wierdak 2016]. Thymol is one of the main substances in the essential oils of *Satureja* plants. It is often reported that thymol levels in plants do not fluctuate drastically during ontogenesis [Mastelić and Jerković 2003, Chorianopoulos et al. 2006, Kosar et al. 2008, Damjanović-Vratnica et al. 2011, Ghotbabadi et al. 2012]. The content of essential oil in the full bloom phase was 2.31% in *Satureja mutica* and 1.45% in *Satureja intermedia*. Carvacrol

(30.9%) and thymol (26.5%) were the main components of the *Satureja mutica* essential oil, whereas the oil in *Satureja intermedia* was dominated by thymol (32.3%) and γ-terpinene (29.3%). Essential oils from *Satureja mutica* and *Satureja intermedia* were found to have not only high thymol content but also a chemical composition similar to oils from *Saturaeja hortensis* and *Satureja montana*. They can therefore be used interchangeably as flavouring agents [Sefidkon and Jamzad 2005].

Marrubium vulgare L.

Investigations of horehound [*Marrubium vulgare* L.] have demonstrated that the developmental phase of the plant determines the composition of its essential oil. Horehound plants in the vegetative phase contained the highest content of β-copaene (44.54%) and bicyclogermacrene (20.06%). The level of these compounds decreased during the flowering period to 24.79% and 9.86%, respectively [Zawiślak 2012b]. A study of E-caryophyllene and germacrene D as the main components of *Marrubium vulgare* L. oil [Zawiślak 2011a] showed a comparable level of these substances in *Marrubium incanum* L. herb oil. Therefore, cultivation of *Marrubium incanum* L. may yield raw material with a similar chemical composition of essential oil to that in *Marrubium vulgare* L. [Zawiślak 2012a].

Conclusion

Essential oils as biologically active substances in plants of the family Lamiaceae give herbal raw materials a characteristic aroma, which is not always similarly perceptible in these plants. Typically, the scent changes during the plant vegetation season. This is associated with a decline in the content of some oil ingredients and an increase in the level of other compounds.

Various investigations of the relationship between ontogenesis and the quantitative and qualitative composition of oil prove that it is possible to target plant cultivation (raw material harvesting) at achievement of the highest quality of raw material. Analyses of the qualitative and quantitative composition of essential oil from Lamiaceae plants allow a conclusion that they are highly variable during the plant vegetation period. Comprehensive knowledge in this field can help to design a cultivation scheme that will yield the desired aroma profile of oil with the highest suitability for use as a natural cosmetic or food-flavouring agent.

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Faza rozwojowa rośliny jako czynnik modyfikujący skład i jakość olejku eterycznego w wybranych roślinach z rodziny Lamiaceae

Streszczenie. Olejki eteryczne są podstawowymi związkami aktywnymi w roślinach z rodziny Lamiaceae. Zawartość olejków w roślinie jest zmienna w okresie wegetacji rośliny. Skład jakościowy i ilościowy olejków eterycznych jest modyfikowany ontogenezą rośliny. Znając tendencję do zmian składu chemicznego poszczególnych olejków, można zaproponować taki termin zbioru, w którym w olejku występuje największe nagromadzenie związków decydujących o silnym zapachu i właściwościach leczniczych. Zaprezentowane w pracy olejki: bazyliowy, hyzopowy, melisowy, cząbrowy są powszechnie znane. Natomiast rzadko spotykany jest olejek z szanty.

Słowa kluczowe: bazylia, hyzop, melisa, cząber, szanta

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Content of active substances, activity, and application of nut oils

Summary. In recent years, the vegetable oil supply and range have expanded by introduction of new products that were previously unavailable on the Polish market. These include oils pressed from hazelnuts or walnuts, which are grown in Polish climate conditions, and exotic nuts e.g. macadamia, pecan, or Brazil nuts. Vegetable oils, including nut oils, are used in the pharmaceutical, cosmetic, and nutrition industries. They are highly value due to the high content of essential fatty acids, whose deficiency in the organism results in skin problems, loss of immunity, and liver, kidney, or heart diseases. Due to the high content of bioactive substances such as tocopherols, phytosterols, polyphenol compounds, vitamin E, group B vitamins, and minerals, the oils are a valuable component of cosmetic recipes, in particular for production of creams, lotions, balms, and conditioners. The present review based on the available literature data describes the chemical composition and application of oils from various types of nuts.

Key words: Brazil nuts oil, hazelnut seed oil, walnut seed oil, macadamia seed oil, essential fatty acids

Introduction

Vegetable oils are fats extracted from plants. They have a liquid consistency at room temperature, with the exception of coconut oil, palm kernel oil, and palm oil, which are solid oils. In chemical terms, they are a mixture of glycerine and higher fatty acid esters. Vegetable oils have been used in many areas of life, e.g. nutrition, dietetics, medicine, cosmetology, tribology, and as alternative fuel sources [Szczypska 2019]. They are highly valued as nutritional products due to their high vitamin content, energy value, and the presence of essential fatty acids (EFAs), which are important for proper development and function of the human organism [Kłopotek et al. 2017]. EFA deficiencies in the diet and impaired absorption or abnormal metabolism in the human organism can be one of the causes
of many serious diseases, e.g. atherosclerosis, cardiovascular diseases, and

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brain or skin conditions [Lamer-Zarawska 2015]. Since ancient times, vegetable oils have also been applied in cosmetology as a basis of many cosmetics. Current cosmetic recipes are frequently based on vegetable oils due to their biological properties and role of carriers of other active substances [Michalak et al. 2018]. EFA contained in vegetable oils exert a highly beneficial effect on the skin and exhibit favourable absorption and anti-allergic properties. Their deficiency may result in excessive desquamation of the epidermis [Lamer-Zarawska 2015]. Furthermore, vegetable oils used as a base in the production of cosmetics cover the epidermis with a thin protective layer, which effectively prevents water loss and maintains skin elasticity and firmness. Additionally, they soften the stratum corneum and reduce skin tension and pain or itching related to inflammation [Bojarowicz and Woźniak 2008]. Vegetable oils are an excellent solvent for many compounds with high biological activity, including vitamins (A, D, and E) and vegetable pigments (carotenoids and chlorophylls). However, the content of these active substances in vegetable oils depends on the species, maturity of the raw material, and extraction technology [Rotkiewicz et al. 2002]. Plant oils are extracted from various parts of plants, including fruits (e.g. olives, sea buckthorn), seeds (e.g. borage, flaxseed, sea buckthorn), fruit stones (e.g. grapes, blackcurrant, raspberries, plums), nuts (e.g. walnut, hazelnut), or sprouts (e.g. wheat) [Obiedzińska and Waszkiewicz-Robak 2012, Michalak and Kiełtyka-Dadasiewicz 2018]. Cold-pressed oils are mostly valued for their nutritional value and stability [Kłopotek et al. 2017, Michalak and Kiełtyka-Dadasiewicz 2018]. Nut oils are extracted from various species of plants whose fruits are nuts. Sometimes, in botanical terms, they are drupes whose stones are called nuts. They are a natural source of fatty acids with low content of saturated fatty acids (SFAs) and high content of unsaturated fatty acids predominated by monounsaturated fatty acids (MUFAs) in most nuts [Kris-Etherton et al. 1999, Ros 2010]. The best-known nut oils that are applied in dietetics, pharmacy, and cosmetology include Brazil nut oil, pecan oil, hazelnut seed oil, walnut seed oil, almond oil, and macadamia nut oil [Lamer-Zarawska 2015, Michalak and Kiełtyka-Dadasiewicz 2018].

The aim of the study was to analyse the content of active substances present in selected nut oils and to indicate their application in dietetics, pharmacy, and cosmetology.

Brazil nut oil (*Oleum Bertholletia*)

The Brazil nut tree (*Bertholletia excelsa* Humb.) of the family *Lecythidaceae* can be found in several Latin American regions, especially in the Amazon Basin. It has considerably large potential use and is considered one of the major Amazon oleaginous seeds [Santos et al. 2012]. Brazil nuts are known all over the world for their enormous nutritional value [John and Shahidi 2010]. On an

industrial scale, oil from seeds is extracted by hot or cold old extraction using a hydraulic or mechanic press after previous thermal treatment of the nuts [Santos et al. 2012]. The total content of oil in the seeds is around 60–70 g 100 g^{-1} [Ryan et al. 2006, Queiroga Neto et al. 2009]. The pale yellow oil with the characteristic aroma and flavour contains 15% of SFAs, including palmitic and stearic acids. Additionally, there are 5% of MUFAs in Brazil nut oil, including large quantities of oleic acid and 21% of PUFAs, predominantly linoleic acid [Muniz et al. 2015]. Brazil nut oil also contains tocopherols (α-tocopherol – 72.55 μg g^{-1}, γ-tocopherol – 74.35 μg g^{-1}, δ-tocopherol – 5.90 μg g^{-1}), phytosterols (β-sitosterol – 79.00 mg $100g^{-1}$, campesterol – 4.00 mg 100 g^{-1}, stigmasterol – 11.33 mg 100 g^{-1}, brassicasterol – 1.50 mg $100g^{-1}$), polyphenols, and squalene [Costa et al. 2010]. They also contain magnesium, calcium, selenium, cooper, iron, potassium, zinc, phosphorus, niacin, and vitamins E, B_1, and B_6 [Santos et al. 2013]. Due to their content of bioactive components, including those with antioxidant properties, Brazil nuts help to reduce the incidence of cardiovascular diseases and eliminate risk factors such as oxidative stress, inflammation, high cholesterol, and diabetes [Cardoso et al. 2017]. Brazil nut oil is often used in the confectionery industry and as one of the best technical oils for e.g. lubrication of metal parts of various devices, e.g. the inner elements of traditional watches [Lamer-Zarawska 2015]. With its high content of oleic (ca. 55%), palmitic (13.5%), and linoleic (21%) acid glycerides, Brazil nut oil can be used in the cosmetics industry [Michalak and Kiełtyka-Dadasiewicz 2018]. Brazil nuts have the highest content of squalene (1377.8 mg/g oil), i.e. a tripeptide hydrocarbon with antioxidant properties. Its physiological role in the organism consists in support of oxygen transport to cells, removal of xenobiotics, and involvement in sterol metabolism. Moreover, it plays a role in the synthesis of bile acids, sex hormones, and provitamin D [Januszewska-Jóźwiak and Synowiecki 2008].

Hazelnut seed oil

The hazelnut (*Corylus avellana* L.) from the family *Betulaceae* is a popular nut in the world. It is grown at the Black Sea coast in Turkey, in Southern Europe (Italy, Spain, Portugal, and France), in some parts of the US (Oregon and Washington), and in temperate climate regions of the northern hemisphere [Topkafa et al. 2019]. The most important nutrients in hazelnuts are oils (54.6–63.2%) containing oleic acid (39.5%), palmitoleic acid (37.0%), linoleic acid (6.9%), eicosaenoic acid (4.6%), docosenoic acid (3.4%), eicosanoic acid (4.6%) and smaller contents of palmitic, linolenic, stearic, and tetraeicosanoic acids [Savage et al. 1997]. Hazelnuts are also a valuable source of vitamins (vitamin B_6), minerals (magnesium, potassium, calcium, manganese, iron, zinc, phosphorus, and copper), polyphenols and tocopherols (α-tocopherol – 22.90 mg $100g^{-1}$, β-tocopherol – 0.50 mg $100g^{-1}$, ɤ-tocopherol – 1.10 mg $100g^{-1}$) [Deon et al. 2018]. Additionally, they are appreciated

for their high nutritional value. Besides the compounds listed above, hazelnuts contain 16% of protein and 3.5% of sugar; hence, they are widely used in the confectionery industry. There are a few different techniques for extraction of hazelnut oil, e.g. mechanical screw pressing, cold pressing, supercritical fluid extraction, microwave, and organic solvent extraction. The cold pressing technique is less efficient than the other methods; however, cold-pressed seed oils have high nutritional value and important chemical properties, because the raw material is not exposed to a heat treatment, which causes loss of nutrients [Topkafa et al. 2019]. Nuts and extracted oil are often recommended as a part of healthy diet due to their strong antioxidant properties related to their high content of polyphenols (291.00 mg of GAE $100g^{-1}$ f.w.) and tocopherols (38.40 mg $100g^{-1}$) [Kornsteiner et al. 2006]. It has also been suggested that the inclusion of hazelnuts in the diet as a source of MUFAs is associated with favourable plasma lipid profiles and reduced risk of coronary heart disease [Mercanligil et al. 2007]. Hazelnut oil is frequently used in the cosmetics industry, since it perfectly nourishes and revitalises all skin types. It also has stabilising and astringent properties; therefore, it is recommended for oily, seborrhoeic, acne, and couperose-prone skin. The oil is highly valued, as it is rapidly absorbed by the skin and does not leave a greasy film on its surface. It is a common ingredient in bath cosmetics, massage products, and day care creams [Lamer-Zarawska 2015].

Walnut seed oil (*Oleum Juglandis*)

The walnut (*Juglans regia* L.) is a plant of the family *Juglandaceae* native to central Asia, the Western Himalayas, and Kyrgyzstan [Salas-Salvadó et al. 2011]. It was introduced in Europe before Roman times, from where it spread to other regions with a Mediterranean type ecosystem throughout the world such as the USA and North Africa. Nowadays, it is in continuous expansion thanks to new cultivars that adapt better to different climatic conditions. Currently, China is the major crop producer followed by the USA, Iran, and Turkey [Fregapane et al. 2019]. Walnut seeds contain 54–72% of oil (depending on the variety and cultivation condition), 25% of protein rich in essential amino acids, and 12–16%
of carbohydrates. Additionally, walnut seeds contain cellulose, minerals and polyphenols [Poggetti et al. 2018, Fregapane et al. 2019]. Walnut oil contains SAFs, i.e. palmitic (6.7–7–8%) and stearic acids (1.5–2.7%), and unsaturated fatty acids, i.e. linoleic (50–65%), α-linolenic (1.9–19.0%), and oleic acids (14.5–28.0%). The bioactive compounds in the oil include tocopherols in total amount around 223–647 mg kg^{-1} (γ-tocopherol, δ-tocopherol, α-tocopherol, β-tocopherol), carotenoids and phytosterols (β-sitosterol, campesterol, δ-5-avanesterol) [Fregapane et al. 2019, Michalak and Kiełtyka-Dadasiewicz 2019]. Walnut oil should not be used for frying due to its susceptibility to oxidation. Additionally, at high cooking

temperatures, it loses its health-enhancing properties and becomes bitter [Kłopotek et al. 2017]. Virgin walnut oil is used for consumption, mainly as salad dressing, and in the cosmetic industry as a component of dry-skin creams and anti-wrinkle and anti-aging products [Martínez et al. 2010]. The oil also has strong skin softening and penetrating properties. It is an ingredient of repair creams, lotions, hair conditioners, and epidermis-renewal formulations [Lamer-Zarawska 2015]. Walnuts have a beneficial impact on hypertension, diabetes, inflammation, cancer, and other epidemiological and clinical problems. Walnuts are known to be a good source of essential linoleic acid, which decreases LDL-cholesterol and increases HDL-cholesterol [Davis et al. 2007]. Moreover, walnut oil possesses a 4 : 1 ratio of n-6 and n-3 PUFAs, which helps to prevent diseases, but makes it more susceptible to oxidation, which is however balanced by the high content of natural antioxidants [Gharibzahedi et al. 2014].

Macadamia nut oil (*Macadamiae Oleum*)

The Queensland nut (*Macadamia ternifolia* F. Muell) of the family *Proteaceae* is an evergreen native Australian tree cultivated in many regions of the world, mainly in Hawaii, California, Brazil, Kenya, and Costa Rica [Trueman 2013]. Macadamia nuts are considered the tastiest nuts in the world, but they tend to turn rancid within 2–3 months [Lamer-Zarawska 2015]. They are a rich source of nutrients and bioactive compounds. Depending on the variety, seed maturity, location and growth conditions, macadamia nuts vary in their content of lipids (33–65%), protein (8–20%), crude fibre (6–30%), and polyphenols [Navarro and Rodrigues 2016]. Other bioactive components of the oil are tocopherols and sterols [Kaijser et al. 2000]. Macadamia nut oil contains 55–63% of oleic acid, 7–11% of saturated palmitic acid, and 18–22% of unique palmitoleic acid. The nuts are considered the best source of the latter acid, which is also a component of human skin; hence, macadamia oil is perfectly absorbed and tolerated. Additionally, macadamia oil is very resistant to auto-oxidation and has antioxidant and anti-radical activity [Lamer-Zarawska 2015]. It is used for production of various types of cosmetics, e.g. night creams, anti-aging creams, lotions, and massage and sunbathing cosmetics. The large range of macadamia oil applications is ensured by its numerous properties, e.g. skin softening, moisturizing, smoothing, and repairing effects as well as inhibition of aging and protection against radiation. Moreover, macadamia oil is safe for sensitive and delicate skin damaged by external factors [Lamer-Zarawska 2015]. Macadamia oil is additionally used in the kitchen primarily as raw salad and dressing oil. It is also recommended for frying and cooking. Due to the presence of mainly monounsaturated fatty acids in its composition, the oil has a relatively high smoke point, which indicates that it can be used for short-term frying and cooking without losing its distinct flavour and aroma [Kłopotek et al. 2017].

Conclusions

Nut oils are primarily a rich source of fatty acids; therefore, they are successfully used in the pharmaceutical, cosmetic, and food industries. Virgin and cold-pressed oils are the most valuable ingredients, especially in the design of cosmetics and dermocosmetics, as these two oil extraction methods ensure a level of active substances identical to that present in the raw material. With their nutritional value, nuts and nut-derived oils are extremely valuable food products. Among the numerous active substances present in nut oils, the highest cosmetological importance is ascribed to unsaturated fatty acids, in particular omega-6 and omega-3 fatty acids, which represent essential fatty acids. They have a positive effect not only on human health but also on the appearance of skin. In cosmetics, nut oils are used in two ways: as a source of active substances and as a solvent for many natural substances with high biological activity. Therefore, they are often the basic ingredients of cosmetic recipes for regenerating and anti-aging creams, emulsions, lotions, hair conditioners, bath balms, lipsticks, and many others. This explains the great popularity of high-quality vegetable oils, including nut oils that are increasingly available on the market.

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Substancje aktywne, działanie i zastosowanie wybranych olejów z orzechów

Streszczenie. W ostatnich latach poszerzyła się oferta olejów roślinnych o nowe produkty – niedostępne wcześniej na polskim rynku. Są to oleje tłoczone z orzechów laskowych lub włoskich, które są uprawiane w polskich warunkach klimatycznych, a także oleje z orzechów egzotycznych, np. orzechów makadamia, pekan lub brazylijskich. Oleje roślinne, w tym oleje orzechowe, znajdują zastosowanie w przemyśle farmaceutycznym, kosmetycznym i żywieniowym. Cenione są ze względu na wysoką zawartość niezbędnych nienasyconych kwasów tłuszczowych, których niedobór w organizmie powoduje problemy skórne, utratę odporności, choroby wątroby, nerek czy serca. Ze względu na wysoką zawartość substancji bioaktywnych, takich jak tokoferole, fitosterole, związki polifenolowe, witamina E, witaminy z grupy B oraz minerały, oleje te są cennym składnikiem receptur kosmetycznych, w szczególności kremów, lotionów, balsamów i odżywek. Niniejszy przegląd, oparty na dostępnych danych literaturowych, opisuje skład chemiczny i zastosowanie olejów z różnych rodzajów orzechów.

Słowa kluczowe: olej z orzechów brazylijskich, olej z orzechów laskowych, olej z nasion orzecha włoskiego, olej z nasion makadamia, niezbędne kwasy tłuszczowe

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Selected biologically active substances and healing properties of dandelion (*Taraxacum officinale*)

Summary. Due to the lack of documented data on the toxicity of dandelions, the FDA (Food and Drug Administration) and the Council of Europe have recognized it as safe for use in food when the content of liquid or solid extracts does not exceed 0.014% and 0.003%, respectively [Sweeney et al. 2005, Martinez et al. 2015]. Daily allowable dose of dried dandelion roots or leaves is in the range of 4–10 g, while fresh raw material – is about 50 g. According to the British pharmacopoeia, you can consume 0.5–2 g of dried roots daily, 3–5 g of dried leaves daily or drink tincture from the root in an amount of 4–8 ml three times a day or from leaves – 5–10 ml twice a day [González-Castejón et al. 2012]. *Taraxacum officinale* has great healing potential and deserves a wider interest, especially since it is an undemanding plant that can grow in various environmental conditions. Health-promoting properties: anti-inflammatory, antioxidant, anti-diabetic, antibacterial, prebiotic, anti-thrombotic activity anti-cancer or hypolipidemic properties encourage further research [Lis and Grabek-Lejko 2016].

Key words: *Taraxacum officinale*, biologically active substances, healing properties

Introduction

Taraxacum officinale (L.) Weber ex F.H. Wigg is a medicinal herbs and member of *Compositae* family, *Cichorioideae* subfamily, *Lactuceae* tribe [Petkova et al. 2015]. *Taraxacum officinale* is widely distributed in the warmer temperate zones of the Northern Hemisphere (Europe, Asia, and North America) as a perennial plants [Marciniuk et al. 2010, Bajaj 2012]. Schütz reported, the first reference to its application is reflected in its name, which is derived from the Greek words *taraxis* for inflammation and *akeomai* for curative [Schütz et al. 2006]. Dandelion derives from French *dent-de-lion*, meaning literally 'lion's tooth', bring to mind the shape of its leaves [Schütz et al. 2006, Biel et al. 2017]. It has many English common names including: Bitterwort, Irish daisy, Blow-ball, Yellow gowan, Cankerwort, Clockflower, Common dandelion, Piss-in-bed, Pissinlit,

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Priest's crown, Puffball, Telltime [OARDC 2020]. According to Hegi [1987], the genus *Taraxacum* WIGG. includes approximately 57 varieties with many microspecies.

Flower stems of *Taraxacum officinale* arise from a rosette formed by the leaves, are hollow and finished on a bright yellow floral section. Moreover, cutting stem of the plant exudes a sap latescente. Reproduce is by wind-blown seeds and regenerate from root fragments. The species requires moist soil and sunlight in order to establish and thrive [OARDC 2020]. The multidirectional effect of dandelion indicates its potential importance in the prevention and therapy of certain diseases. *Taraxacum officinale* generally studied as a weed, has a wide range of chemical components which are responsible for its healing properties [Lis and Grabek-Lejko 2016].

The present study provides information about biologically active substances of *Taraxacum officinale* (L.) Weber ex F.H. Wigg, based on the available literature.

Biologically active substances

Natural herb, *Taraxacum officinale,* have been used as a phytomedicine. Among others, Amin et al., suggested that the *Taraxacum officinale* active ingredients are found in the leaves and roots [Amin et al. 2013]. The large, green (light to dark) leaves (5–40 cm long) are clustered in a rosette at the base of the plant and are deeply serrated [Schütz et al. 2006].

Accordingly, the leaves of *Taraxacum officinale* has drawn the attention of researches an understanding of the healing properties [Noori et al. 2012]. The most important biologically active components are sesquiterpenic lactones, biotin, inositol and vitamins B, D, E and phosphorous (P). The most detailed list of active substances derived from the leaves and roots of dandelion was described by Schütz et al. [2006] and Wirngo et al. [2016]. The chemical composition of dandelion depends on many factors, including from season, harvest time, ecological factors. The number and type of chemical compounds vary significantly between leaves, roots and flowers [Lis and Grabek-Lejko 2016.]

Root components described by Wirngo et al. [2016]: Taraxasterol (anti-inflamatory properties, Caffeic acid, Ixerine, Chicoric acid, Ainsloside, Tetrahydroridentin B, Monocaffeoyltartaric acid, 11β,13-dihydrolactucin, Taraxacolide β-D glucoside, Taraxinic acid β-D glucoside. The roots of *Taraxacum officinale* contain carotenoids (e.g. lutein), carbohydrates (e.g. inulin), minerals, sugars (e.g. glucose, fructose, and sucrose), choline, vitamins, fatty acids (e.g. myristic acid), mucilage, and pectin. Nearly 45% of the roots consist of inulin with many beneficial effects such as the elimination of pathogens in the gastrointestinal tract, obesity, osteoporosis and cancer [Roberfroid 1999].

Schütz et al. suggested that the, *Taraxacum officinale* roots has been utilized for the treatment of various ailments such as dyspepsja, kidney disease, liver complaints, heartburn, spleen, hepatitis [Schütz et al. 2006].

Flower components described by Wirngo et al. [2016]: Caffeic acid, Chlorogenic acid, Chrysoeriol, Luteoline 7-O-glucoside, Chicoric acid, Monocaffeoyltartaric acid. These constituents are antimutagenic, anti-inflammatory, and can activate the body's endogenous antioxidant systems [Hu and Kitts 2003].

Dandelion flowers are rich in carotenoids and xanthophylls (oil-soluble antioxidants) including lutein and zeaxanthin, which have a well-established role in protecting the *macula lutea* (yellow spot – it's yellow because it accumulates these compounds) of the retina against UV damage and the potential development of macular degeneration.

Leaf and stem components described by Wirngo et al. [2016]: β-sitosterol, α-amyrin, stigmasterol, quercetine glycosiders, monocaffeoyltartaric acid, sesquiterpene lactones, chicoric acid. The chemical compounds present in the leaves are mainly bitter sesquiterpene lactones, polyphenols and coumarins. In addition, *Taraxacum officinale* leaves are rich in vitamins, especially vitamin A, whose concentration is even higher than in carrots [García-Carrasco et al. 2015, Lis and Grabek-Lejko 2016].

Healing properties

According to Petkova et al. [2015] and Schütz et al. [2006] the healthy-effect of *Taraxacum officinale* root were attributed to their high phenolic content, mainly hydroxycinnamic acid derivatives and flavonoids and a strong relationship between antioxidant activity and phenolic content in the extracts for dandelion roots. The presence of inulin in dandelion roots additionally increase their biological activity and will improve the health benefits for human nutrition [Schütz et al. 2006].

In the following, the scientific investigations supporting the pharmacological properties ascribed to Taraxacum [based on Schütz et al. 2006]:

Diuretic activity – dandelion is helpful in urinary tract infections, bladder diseases, kidneys diseases, gallbladder and *urolithiasis*. The results described by Schütz et al. [2006] showed that „the diuretic action of extracts obtained from dandelion herb was consistently stronger than that from the root extracts, reaching the highest diuretic and saluretic indicescorresponding to 8 g dried herb/ kg body weight" [Schütz et al. 2006, Clare et al. 2009].

Choleretic activity – Böhm [1959] reported a pronounced „increase in bile production in rats after intraduodenal administration of an alcoholic *Taraxacum officinale* leaf extract. In contrast, an aqueous leaf extract was ineffective in the same test model" [Schütz et al. 2006]. The plant improves secretion bile and

facilitates its flow. Main properties exhibits sesquiterpene lactone, which causes irritation of the nerve end of the tongue back, stimulating salivation and increasing secretion digestive juices.

Taraxacum officinale is known for a long time and the secretion used pepsin and hydrochloric acid in the stomach, and secondary influencing also for bile secretion. Abused is also a liver disease in the treatment of cirrhosis liver and after viral inflammation liver [Ezhilarasan 2016, Mahboubi and Mahboubi 2020]. The hepatoprotective effect of dandelion extract (0.5 mg/ml) was confirmed by histopathological examination. Normal hepatocyte system, partial reduction of median vascular dilation and smaller inflammatory infiltrates were observed, which reduces the risk of developing liver parenchymal disease [Colle et al. 2012].

Moreover, *Taraxacum officinale* leaf extracts have been proven to inhibit pancreatic lipase activity in vitro and in vivo by 86.3% and 95.7%, and can also be used as an anti-obesity agent. In this study, Zhang "investigated the inhibitory activity of *T. officinale* against porcine pancreatic lipase *in vitro*. At a concentration of 250 μg/ml, the *T. officinale* extract exhibited 90.2% of the inhibition activity of Orlistat against pancreatic lipase, and *T. officinale* inhibited pancreatic lipase in a dose-dependent manner with an IC50 of 78.2 μg/ml" [Zhang et al. 2008].

Anti-inflammatory activity – Kim [Kim et al. 2000] studied the effect of a *Taraxacum officinale* leaf extract on the production of TNFα (tumor necrosis factor *alpha*) from primary cultures of rat astrocytes stimulated with substance P and lipopolysaccharide. The extract administered concentrations of 100 and 1000 μg/mL significantly supressed TNFα production by inhibiting interleukin-1 production. Respectively, the authors suggested an anti-inflammatory activity of dandelion leaf extract in the CNS (central nervous system) [Schütz et al. 2006]. Research reported by Awortwe et al. [2011], aimed at investigating the anticholinergic effect of the *Taraxacum officinale* ethanol extract and its effect on some inflammatory cells of the trachea caused by ovalbumin. An *in vivo* experiment was performed on guinea pigs. The obtained histological results confirm the very good effectiveness of the leaf extract in the treatment of inflammation [Awortwe et al. 2011].

Anti-oxidative activity – leaf extracts contain about three times more phenolic compounds (9.9%) and flavonoids (0.086%) than root extracts [González--Castejón et al. 2014].The antioxidant properties of dandelion are determined by the presence of many ingredients. The most effective antioxidants are polyphenolic compounds. Their total content in the aerial parts of the plant is 15.5 mg/g dry matter [Sengul et al. 2009] and in the root – 9.491 mg/g dry matter [Kenny et al. 2014]. Schütz reported that „due to the higher polyphenol content, the leaf extract was a more effective hydrogen donor, reducing agent and hydrogen peroxide scavenger compared to the root extract" [Schütz et al. 2006]. "The highest DPPH

radical scavenging activity (a lower value of EC50 indicates a higher activity) was detected in the leaf extract with an EC50 of 1.9 μg/mL, followed by the root extract with an EC50 of 12.6 μg/mL and the crude powdered root extract with an EC50 of 65.0 μg/mL. This is consistent with the highest total phenollevels. Compared to pure compounds, its antioxidant activity is similar to that of pure polyphenols such as catechin (2.17 ±0.11 μg/mL), caffeic acid (1.94 ±0.08 μg/mL) and quercetin (1.63 ±0.07 μg/mL). This results are in agreement with previous activity found for aerial parts of *Taraxacum officinale* using the DPPH metod" [García-Carrasco et al. 2015].

Anti-carcinogenic activity – Koo [Koo et al. 2004] described „the effects of dried aqueous dandelion (*Taraxacum officinale*) herb extracts on the cytotoxicity and production of cytokines in human hepatoma cell lines (Hep G2). The *Taraxacum officinale* extract caused a time-dependent and partially dose-dependent reduction of cell viability up to 26% (IC50)".

Skin neoplasms (melanomas), usually resistant to available chemotherapy, react to dandelion root extracts that cause apoptosis in these cells, while not negatively affecting non-cancer cells [Chatterjee et al. 2011].

It also supports the treatment of female ailments such as uterine and breast cancers [Choi et al. 2010, Chatterjee et al. 2011].

Anti-allergic activity – Schütz et al. [2006] and Ho et al. [1998] reported a „guaianolide sesquiterpene isolated from *Taraxacum platycarpum* DAHLST, was investigated for its anti-allergic activity by measuring the release of β-hexosaminidase from rat basophilic leukemia (RBL-2H3) cells, which occurs concomitantly with the release of histamin when mast cells are immunologically activated". The resutts of this study suggested "that anti-allergic activity of β-hexosaminidase relase from RBL-2H3 cells was far more potent than that of disodium cromoglycate witchwitch is actually used in clinical trials IC50 value of DSCg is estimated over 100 μM".

Anti-hyperglycemic activity – Petlevski et al. [2001] demonstrated an „antihyperglycemic effect of a herbal preparation containing 9.7% *Taraxaci radix* (*Taraxacum officinale* WEBER). For its production, the dry plant material was extracted with 60% etanol".

Anti-coagulatory/anti-thrombotic activity – Neef et al. [1996] described that „the ethanolic extracts of dandelion (*Taraxacum officinale* WEBER) root were investigatedfor their inhibitory effects on human platelet aggregation. The extracts caused a dose-dependent inhibition of ADP induced aggregation, with a maximal inhibition of 85% observed at a concentration corresponding to 0.04 g dried root/ mL of human platelet-rich plasma (PRP)". Dandelion also reduces the concentration total cholesterol and serum triglycerides and in liver cells as cholesterol increases in the HDL fraction [Majewski et al. 2020].

Prebiotic activity – Tabasumi et al. [2018] reported that dandelion roots have adistinct demulcent action and prebiotic properties due to their kontent of inulin.

Moreover, Trojanová et al. [2004] reported that „aqueous root extracts of *Taraxacum officinale* WEBER were tested for their growth-stimulating activity of 14 different strains of bifidobacteria. The growth of six strains (*B. adolescentis 1* and *2, B. bifidum 1, B. catenulatum, B. longum 2*) was significantly enhanced in the medium containing dandelion Root extract. Determination of carbohydrates before and after incubation in All *Bifidobacterial* cultures revealed 1–48% utilization of dandelion oligofructans".

Anti-diabetic properties – Dandelion offers a compelling profile of bioactive components with potential anti-diabetic properties: inhibition of renal glucose reabsorption, reduction of the activity of carbohydrate enzymes and stimulates hepatic glycolysis and glycogenesis [Wirgo et al. 2016]. Resnick and Howard [2002] described the main factor in type 2 diabetesis the dysregulation of insulin secretion and insulin sensitivity that leads to hyperglycemia – increased blood sugar levels and type 2 diabetes, which can later cause the development of vascular diseases. Moreover, Mir et al. [2015] described the mechanisms by which plant-derived compounds manifest their anti-diabetic properties: inhibition of renal glucose reabsorption, reduction of dietary blood sugar and the activity of carbohydrate enzymes, inhibition of potassium channel flow.

Antibacterial activities – Ghaima et al. [2013] analyzed the anti-bacterial properties of ethyl acetate extract from *Taraxacum officinale* leaves against Aeromonas *hydrophila, Salmonella typhi, S. aureus, Bacillus cereus* and *E. coli*. Growth inhibition of *S. typhi*, *S. aureus*, *B. cereus* and *E. coli* was inhibited. Antibacterial activity of ethyl acetate extract of dandelion against some of food borne bacteria at 10 mg/ml (diameter of inhibition zone – mm) presented *S. typhi* (14 mm), *S. aureus* and *E. coli* (16 mm), *B. cereus* (18 mm), *A. hydrophila* (no inhibition). Ethanol leaf extract (200 mg/ml) was shown to inhibit the growth of *E. coli* and *S. aureus* (zone of inhibition 23.50 and 10.75 mm) [Oseni and Yussif 2012]

Only *A. hydrophila* did not inhibit the growth zone, which indicates a very high resistance of this strain to phytochemical compounds contained in the extract.

Other medical applications – numerous medicinal properties of dandelion used is to treat skin problems (psoriasis, acne), warts, fungal infections. Dandelion root medical stimulates the appetite and facilitates digestion. Syrup in turn, dandelion is a popular soothing agent cough and supports the treatment of rheumatism. In women lactating increases milk production. Shows also calming properties [Lachowicz and Oszmiański 2017].

Conclusions

Taraxacum officinale has a number of medicinal and therapeutic properties. *Taraxacum officinale* roots has been utilized for the treatment of various ailments such as dyspepsia, kidney disease, liver complaints, heartburn, spleen,

hepatitis. Dandelion flowers are rich in carotenoids and xanthophylls, which have a well-established role in protecting the *macula lutea* of the retina against UV damage and the potential development of macular degeneration. The knowledge and identification of many dandelion components still require research and analysis in order to confirm their interesting properties *in vivo* and in clinical trials.

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Wybrane substancje biologicznie czynne i właściwości lecznicze mniszka lekarskiego (*Taraxacum officinale*)

Streszczenie. Ze względu na brak udokumentowanych danych dotyczących toksyczności mniszka lekarskiego FDA (Food and Drug Administration) i Rada Europy uznały go za bezpieczny do stosowania w żywności, gdy zawartość płynnych lub stałych ekstraktów nie przekracza odpowiednio 0,014% i 0,003 % [Sweeney i in. 2005, Martinez i in. 2015]. Dzienna dopuszczalna dawka suszu z korzeni lub liści mniszka lekarskiego mieści się w przedziale 4–10 g, natomiast surowca świeżego – około 50 g. Według brytyjskiej farmakopei można codziennie spożywać 0,5–2 g suszonych korzeni, 3–5 g suszonych liści dziennie lub pić nalewkę z korzenia w ilości 4–8 ml trzy razy dziennie lub z liści – 5–10 ml dwa razy dziennie [González-Castejón et al. 2012]. *Taraxacum officinale* ma duży potencjał leczniczy i zasługuje na szersze zainteresowanie, zwłaszcza że jest to mało wymagająca roślina, która może rosnąć w różnych warunkach środowiskowych. Właściwości prozdrowotne: przeciwzapalne, przeciwutleniające, przeciwcukrzycowe, przeciwbakteryjne, prebiotyczne, przeciwzakrzepowe, przeciwnowotworowe czy hipolipidemiczne zachęcają do dalszych badań [Lis i Grabek-Lejko 2016].

Słowa kluczowe: *Taraxacum officinale*, substancje biologicznie czynne, właściwości lecznicze

Joanna ŚLUSARCZYK[1]

St. John's Wort (*Hypericum perforatum* L.) as a source of active compounds with a wide range of therapeutic effects

Summary. The paper presents the occurrence and morphological characteristics of St. John's Wort (*Hypericum perforatum* L.) plants. It discusses the major groups of active compounds and their medicinal application. Currently, the most common therapeutic use of St. John's Wort is the treatment of mild to moderately severe depressive disorders. The study also indicates the danger of interactions of the active compounds contained in St. John's Wort formulations with synthetic cardiac, anti-asthmatic, and immunosuppressive drugs, which may reduce their bioavailability.

Key words: active compounds, antidepressant activity, flavonoids, hypericin, St. John's Wort (*Hypericum perforatum* L.)

Introduction

St. John's Wort (*Hypericum perforatum* L.) is a medicinal plant known since antiquity and widely used due to its biological properties. In Polish folk medicine, the plant was called St. John's herb, as it flowers around St. John' Day on June 24. In other regions of Poland, St. John's Wort is called Mother of God's blood, as the flowers of this plant crushed with fingers secrete blood-red sap. Similarly, the names of the plant in other languages refer to folk beliefs, e.g. St. John's Wort in English, Herbeŕ mille trous in French, or Johanniskraut in German [Turek 2005].

The therapeutic properties of St. John's Wort (*Hypericum perforatum* L.) have been known since ancient times. They were described by many ancient scholars e.g. Hippocrates, Theophrastus, Dioscorides, Galen, and Pliny [Bombardelli and Marazzoni 1995]. Paracelsus (1493–1541) claimed that there was no other equally effective herb in the treatment of "crazy fantasies" [Nutt 1997]. Therefore, St. John's Wort has been recommended for centuries as an effective agent for alleviation of respiratory and urinary tract disorders and digestive

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problems as well as treatment of haemorrhoids, injuries, burns, wounds, and various ulcers [Bombardelli and Marazzoni 1995]. Since Paracelsus's time, St. John's Wort has also been used in treatment of neurological and psychological disorders, e.g. neuralgia, anxiety, and depression [Turek 2005].

Characteristics of St. John's Wort (*Hypericum perforatum* L.)

St. John's Wort (*Hypericum perforatum* L.) represents the family *Hypericaceae*. This common plant of the temperate zone occurs naturally in Europe, western Asia, and northern Africa. It is an adventive species in North America, South America, South Africa, Australia, New Zealand, and Japan. In Poland, it usually grows in meadows and roadside localities.

St. John's Wort has a stiff, glabrous, ca. 60 cm high stem branched copiously in its apical part. Its foliage is formed by up to 2 cm long, elliptical or lanceolate apetiolate leaves arranged oppositely on the stem. When viewed against light, they exhibit numerous brighter spots filled with essential oils responsible for the pleasant aroma of the plant. Secretory glands are located on the purple-red leaf margins, especially in the apical part, and partly on the leaf surface. Its actinomorphic flowers with an approx. 3-cm diameter yellow corolla form corymb inflorescences. The fruit is a dehiscent 3-lobed capsule containing numerous small dark brown seeds [Ożarowski and Jaroniewski 1987].

St. John's Wort flowers from June to August. It is harvested twice a year. Its freshly opening heavily foliaged upper parts of the stems up to 25 cm long are the herbal raw material. The apical fragments of stems collected during flowering must be air-dried in thin layers in the shade or in dryers at a temperature lower than 35°C to avoid degradation of biologically active compounds [Ożarowski and Jaroniewski 1987, Głowniak and Widelski 2017]. Until recently, St. John's Wort raw material in Poland was collected in natural localities, but currently it is mainly cultivated, which ensures quality control. Some medicinal plants, including St. John's Wort, are hyperaccumulators of relatively high concentrations of heavy metals (e.g. Cd) in their cells. The accumulation of cadmium in these plants excludes their use as a material for manufacture of natural medicinal products. The increasing environmental pollution poses a potential threat to consumer health [Clemens 2006]. Currently, research is being conducted to determine the effects of elevated cadmium content on the active substances in St. John's Wort [Ślusarczyk et al. 2014].

Basic active compounds in St. John's Wort herb (*Hypericum perforatum* L.)

St. John's Wort has a very rich chemical composition. The abundant active compounds contained in its herb belong to several groups, e.g. naphthodiantrone compounds such as hypericin, pseudohypericin, protohypericin, and isohypericin [Barnes et al. 2001, Greeson et al. 2001, Głowniak and Widelski 2017]. Flowering plants contain approximately 30% of hypericin protoforms, with the highest content in buds (48%) and flowers (30%) and the lowest level in leaves (17%). The lowest content of protopigments in leaves is detected at high solar exposure. The process of transformation of protoforms into respective pigments depends on the radiation wavelength, radiation intensity, and nitrogen content in the air [Briskin et al. 2000, Southwell et al. 2001]. The highest transformation rate is noted during the flowering period at the highest light availability. The rate is even higher when the raw material is dried at light exposure and during extraction. In the natural state, the rate of transformation of protopigments into pigments is probably lower due to the presence of phenolic compounds, which are natural photoprotectors [Poutaraud et al. 2001]. The total hypericin content depends on the date of harvest, plant habitat, mode of drying and storage of herb, and other factors [Southwell et al. 2001]. The content of hypericin in *Hypericum perforatum* L. is in the range of 0.05–0.15% [Kitanov 2001].

St. John's Wort also contains the phloroglucinol derivatives, such as hyperforin (2–4%) and its derivatives. These derivatives, hyperforin, adhyperforin, and furohyperforin, are a large group of compounds with high importance for the biological activity of St. John's Wort. The discovery of hyperforin in 1975 initiated comprehensive research on its biological activity [Maisenbacher et al. 1992, Fukuyama et al. 1993, Rucker et al. 1995, Erdelmeier 1998, Trifunović et al. 1998, Orth et al. 1999, Verota et al. 1999, 2002, Vugdelija et al. 2000, Jensen et al. 2001, Shan et al. 2001]. St. John's Wort has also be found to contain flavonoids (from 2 to 5%), e.g. hyperoside, rutoside, quercetin, and isoquercetin as well as biflavonoids (up to 0.14%), e.g. amentoflavone and biapigenin [Greeson et al. 2001, Henderson et al. 2002 Jensen et al. 2001]. Catechin tannins (up to 8%) such as catechin, epicatechin, and procyanidin B2 are also present in the plant as well [Butterweck and Schmidt 2007].

Additionally, St. John's Wort contains phenolic acids, such as caffeic acid, chlorogenic acid, and neochlorogenic acid, which may be present in high amounts (400 μg/g) depending on the raw material [Derkach and Starikova 2019]. Phenolic acids contained in St. John's Wort also include p-coumaric, ferulic, isopheric, p-hydroxybenzoic, and vanillic acids [Bombardelli et al. 2002, Lamer--Zarawska et al. 2007, Głowniak and Widelski 2017]. The plant also contains xanthones (γ-pyrone derivatives – tetrahydroxyxanthone), which are characteristic of the *Hypericaceae* family, and essential oils (0.05–0.9%) – germacrene D, (*E*)-cary-ophyllene, 2-methyloctane, α-pinene, bicyclogermacrene

and (*E*)-β-ocimene [Barnes et al. 2001, Butterweck and Schmidt 2007, Dordević 2015].

Therapeutic activity of St. John's Wort (*Hypericum perforatum* L.)

Given the abundance of components representing many groups of natural compounds, St. John's Wort herb exerts multifarious effects on the human organism. The type of the activity depends on the chemical composition of the extract determined by the type of solvent used in the extraction process. Water extracts and infusions contain flavonoids, biflavonoids, catechin tannins, and phenolic acids. They exert an astringent and spasmolytic effect on the smooth muscles of the bile ducts, intestines, and urinary tract, seal small blood vessels, and inhibit oxidative processes in these vessels [Lamer-Zarawska et al. 2007]. The relaxant activity of flavonoids facilitates bile flow to the duodenum and prevents bile stagnation in the gallbladder, thereby impeding gallstone formation. Flavonoids (mainly hyperoside) have diuretic activity and increase daily urine output by approximately 20%. This supports removal of metabolic products and prevents deposition of kidney stones. Catechin tannin-containing water extracts have been reported to have antibacterial activity [Ożarowski and Jaroniewski 1987, Dall'Agnol et al. 2003, Głowniak and Widelski 2017]. They also exert an astringent and anti-inflammatory effect on the gastrointestinal mucosa. Additionally, catechin derivatives inhibit minor haemorrhages and seal capillary walls [Ożarowski and Jaroniewski 1987].

Oil or alcohol solutions and powdered St. John's Wort herb contain lipophilic anthra-compounds (naphthodianthrones) such as hypericin, pseudohypericin, and protohypericin. Their red colour is visible after crushing the flowers of this plant. Xanthines, phloroglucinol derivatives (hyperforin and adhyperforin), and essential oil have been detected in oil and alcohol solutions. They exhibit sedative and antidepressant effects and reduce anxiety, irritability, and nervous tension [Głowniak and Widelski 2017]. In turn, oil extracts from fresh flowers (*Oleum Hyperici*) have been reported to have anti-inflammatory activity and promote wound and burn healing. Oil and water-alcohol extracts are used to treat hard-to-heal wounds. Hyperforin is also an antibacterial agent. Naphthodianthrones contained in St. John's Wort herb have antiviral properties. In turn, carotenoids, chlorophyll, and phloroglucinol derivatives stimulate wound granulation.

The antidepressant and sedative effect of St. John's Wort is mainly attributed to the presence of hypericin [Lamer-Zarawska et al. 2007], a red naphthodianthrone pigment, and hyperforin [Mennini and Gobbi 2004]. Alcohol and dry extracts from St. John's Wort improve mood, strengthen, immunize against stress, and have adaptogenic effects. Additionally, they improve learning, remembering, and ability to reproduce information. They also help to restore the

willingness to live. The mechanisms of this action are not fully elucidated. As demonstrated in some studies, alcohol extracts from St. John's Wort inhibit the reabsorption of serotonin, dopamine, and noradrenaline neurotransmitters as well as gamma-aminobutyric acid (GABA) and 1-glutamates by binding to the GABA-A and GABA-B receptor complex. The antidepressant effect is also attributed to xanthines, i.e. MAO-1 inhibitors, and amentoflavone, which binds specifically to diazepam receptors [Lamer-Zarawska et al. 2007, Muszyńska et al. 2015]. Another effect of St. John's Wort formulations is associated with improvement of digestion and stimulation of metabolism, with evident results after prolonged and regular use. Recent investigations of the properties of the active substances contained in the species have reported their antiviral and anticancer effects [Mirmalek et al. 2016]. Hypericin contained in St. John's Wort has anti-inflammatory properties as an inhibitor of stress-activated JNK (c-Jun N-terminal kinases), as demonstrated in investigations of rat peritoneal macrophages [Lee et al. 2008]. The potential application of hypericin is also tested in preclinical studies in animal models of Parkinson's disease [Kiasalari et al. 2016]. At low concentrations, hypericin acting as a photosensitizer induces singlet oxygen-mediated apoptosis. At high concentrations, it tends to cause necrosis with involvement of other reactive oxygen species [Ehrenberg et al. 1998, Dyrała et al. 2015]. With these properties, hypericin is employed as a photosensitizer in photodynamic therapy of cancer. Promising effects have already been achieved in treatment of skin, nasopharynx, bladder, kidney, and pancreas tumours, radiation-induced fibrosarcomas, and haematological and paediatric cancer diseases [Dyrała et al. 2015, Łuczak et al. 2018].

Side effects and interactions with drugs

The intake of St. John's Wort-based formulations may trigger interactions of their active compounds with synthetic drugs. The interactions are associated with the enhancement of the activity of the cytochrome P450 enzyme system induced by herbal active compounds. They accelerate the metabolism of many drugs and worsen their therapeutic effect [Henderson et al. 2002, Russo et al. 2014, Głowniak and Widelski 2017]. Hypericin contained in St. John's Wort herb is a photosensitizing agent. It increases skin sensitivity to sunlight; hence, hypericin-containing formulations should not be used in summer. Overdose or chronic use of St. John's Wort preparations may cause redness, skin burns, internal bleeding, and general asthenia [Ożarowski and Jaroniewski 1987]. Other side effects are associated with gastrointestinal disorders, fatigue, and sleep disturbances [Lamer-Zarawska et al. 2007]. Compounds contained in St. John's Wort formulations interact with such drugs as digoxin (cardiac medication), theophylline (antiasthmatic agent), and cyclosporine (immunosuppressive drug), reducing their bioavailability. They also interact with warfarin, phenprocoumon, and anticonvulsants.

Similarly, products containing St. John's Wort should not be used with MAO inhibitors and selective serotonin reuptake (SSRI) inhibitors (antidepressants). They may also exert adverse effects on anti-cancer and anti-retroviral drugs [Lamer-Zarawska et al. 2007, Łuczak et al. 2018]. Furthermore, due to their content of hyperforin, St. John's Wort extracts may interact negatively with other drugs metabolised by CYP3A4, as the compound induces CYP3A4 in the intestines and liver. In turn, hypericin may induce P-glycoprotein, thus contributing to increased drug resistance [Zhou et al. 2004, Rogowska and Giermaziak 2018]. As shown by Frye et al. [2004], combined application of St. John's Wort formulations and imatinib (a tyrosine kinase inhibitor used in the treatment of e.g. chronic myeloid leukaemia) may lower the effectiveness of the treatment via enhancement of the elimination of imatinib [Szałek et al. 2010].

Conclusion

St. John's Wort (*Hypericum perforatum* L.) is a plant with multifarious therapeutic effects. For centuries, it has been successfully used in treatment of many diseases. Most St. John's Wort formulations available on the Polish and European markets are water-ethanol extracts or dry extracts in the form of tablets or capsules. Due to their effectiveness, they are very popular among patients, and extensive research aimed at elucidation of their properties is being conducted. Recent studies have demonstrated interactions between the common St. John's Wort formulations and anti-cancer drugs. The interactions may exert beneficial or adverse effects on therapy, i.e. either increase the effectiveness of the implemented treatment or enhance side effects. Therefore, further *in vitro* and *in vivo* investigations are recommended for full elucidation of the mechanisms of action of the active compounds contained in St. John's Wort and effective application of these agents.

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Dziurawiec zwyczajny (*Hypericum perforatum* L.) źródłem związków czynnych o szerokim działaniu terapeutycznym

Streszczenie. W pracy przedstawiono występowanie i charakterystykę morfologiczną rośliny dziurawca zwyczajnego (*Hypericum perforatum* L.). Omówiono najważniejsze grupy związków czynnych i ich wykorzystanie w medycynie. Aktualnie najczęstszym działaniem terapeutycznym dziurawca zwyczajnego jest leczenie łagodnych do umiarkowanie ciężkich zaburzeń depresyjnych. Wskazano także na niebezpieczeństwo interakcji związków czynnych zawartych w preparatach z dziurawca z lekami syntetycznymi, o działaniu nasercowym, przeciwastmatycznym i immunosupresyjnym, powodujące obniżenie ich biodostępności.

Słowa kluczowe: związki czynne, działanie antydepresyjne, flawonoidy, hiperycyna, dziurawiec zwyczajny (*Hypericum perforatum* L.)

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Plant and fungal polysaccharides – substances with importance for human health and life

Summary. The paper presents the characteristics of the most important plant and fungal polysaccharides, i.e. starch, cellulose, plant mucilages, and algal fucoidan. Their importance in nature and in human life is discussed, and their application in the pharmaceutical and cosmetic industries is indicated. Many fungal species exhibit the capability of biosynthesis of bioactive compounds. They produce e.g. polysaccharides with anticancer and immunostimulatory activity. The therapeutic properties of secondary metabolites produced by the selected species and their potential application are specified in the paper.

Key words: fucoidan, fungal polysaccharides, plant mucilages, plant polysaccharides

Introduction

Polysaccharides are high molecular weight chemical compounds. A single polysaccharide molecule is made up of many simple sugar molecules linked together by α or β glycosidic bonds. They may have a linear or branched structure [Kączkowski 1993, Tomasik 2009]. Natural polysaccharides play an important role in nature and human life.

Economic importance is primarily assigned to such polysaccharides as cellulose, starch, pectin, and hemicellulose, with starch as the most important of these compounds. Its functional properties and the character of starch-derived products depend on the raw material from which starch is extracted. Other known polysaccharides are vegetable gums, which primarily serve as adhesive agents (glues) and texturing agents in the food industry [Tomasik 2009].

Natural polysaccharides are widely used in the pharmaceutical and cosmetic industries. In medicine, they serve as laxatives or appetite suppressors in the treatment of obesity. Additionally, they exhibit protective and coating potential, and can be used as blood-substitutes. These compounds are also components of

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cosmetic industry products. Polysaccharides with their immunoregulatory and anticancer activity have been intensively studied in recent years, as they are gaining increasing importance in medicine. Polysaccharide compounds, in particular those of fungal origin, are becoming the main components of new drugs with great therapeutic potential. Over 600 fungal species, mainly representatives of *Basidiomycota*, are classified as medicinal mushrooms, as they produce bioactive compounds. They are characterized by a high capacity of biosynthesis of compounds with a strong pharmacological effect [Smith et al. 2002, Rodrigues et al. 2011, Turło 2013].

Plant polysaccharides and their health-enhancing activity – selected examples

Starch is the most common plant polysaccharide. Reserve starch in plants is in the form of diverse granules with various species-specific sizes and shapes. This biopolymer is composed of α-D-glucose molecules. It is a highly important element of human diet, satisfying up to 70% of the energy demand of the entire human population. Amylose and amylopectin are the components of starch. Depending on the plant species, starch comprises approximately 10–30% of amylose and 70–90% of amylopectin. The crystalline structures of starch granules used in cosmetics consist of amylopectin composed of 9 000–10 000 glucopyranose molecules forming a branched chain. In turn, amylose, i.e. a linear polysaccharide polymer made of 300–4000 glucopyranose molecules, is the amorphous phase. Starch is not soluble in cold water; instead, it swells and forms a hydrocolloid structure. Starch is extracted for the cosmetic industry from potatoes, corn, rice, wheat, and oats. It is used as a thickener and co-emulsifier in cosmetic formulations. Starch granules are also used as fillers for liquids and powders, due to the ability to absorb sebum and moisture from the skin, which becomes smooth and soft after application of starch-containing cosmetic products [Głuszek 2015].

Water-insoluble **cellulose** with its derivatives is another natural biopolymer. Cellulose is a structural polysaccharide with a widespread distribution in the world of plants as the main structural component of the cell wall in terrestrial plants. It is also present in some aquatic plants and can be produced by some bacteria, e.g. *Bacillus xylinum*. Almost pure cellulose is extracted mainly from these bacteria and from cottonseed fibres (*Gossypium sp.*), which contain almost 98% of the compound. In other plants, cellulose is present in association with lignin or hemicelluloses (xylans and glucomannans). The cellulose chain structure consists of many β-glycosyl residues connected by a β-1→4-glycosidic linkage. The level of polymerisations amounts to 10 000 but may also exceed 15 000 [Kączkowski 1993]. Cellulose is also important for human health. It is a component of dietary fibre (water-insoluble fraction) and plays a role of a ballast material in the large intestine, facilitating the multiplication of bacterial flora. Cellulose

binds water in the gastrointestinal tract, where it swells and increases its volume, thus facilitating intestinal peristalsis. It exerts a detoxifying effect and reduces excess water in the organism.

Plant mucilages are other important representatives of polysaccharides. They are a combination of simple sugars and uronic acid, which swell in contact with water to form colloidal solutions characterised by high water-binding capacity. Plant mucilages are formed during normal plant growth and are not secreted. In plants, they act as reservoirs of reserve compounds and water and form a protective colloid. They are divided into extracellular, intracellular, and membrane mucilages according to their different locations. Due to their properties, mucilages from many plant species are used in pharmaceutical, cosmetic, and food industries. With their ability to absorb water and form colloid solutions and gels, they are widely applied in medicine, primarily as coating, protective, and softening agents. In recent years, mucilages have been intensively studied to determine their immunoregulatory and antitumour activity. Raw materials from e.g. marshmallow root and flowers, flax seeds, Icelandic lichen, fenugreek seeds, mallow flowers and leaves, linden inflorescences, comfrey root, and mullein flowers are rich in mucilage. For instance, mucilage present in the epidermis of quince seeds (*Cydonia oblonga* L.) accounts for approximately 22% of their weight and is mostly soluble in water. It is composed of arabinose and xylose with a small amount of uronic acids. Formulations containing isolated mucilage alleviate mild inflammation [Kączkowski 1993].

Flax (*Linum usitatissimum* L.) seeds contain 5–8% of mucilaginous substances in the epidermis of the seed coat. Flax seed mucilage is used in medicine as a mild laxative and an ingredient of cataplasms and anti-inflammatory ointments. It is characterised by high water-absorption capacity and functional properties similar to those of gum Arabic. Due to these properties, it is used in the food industry as gum [Czech et al. 2012].

Mucilaginous substances are extracted on an industrial scale from fenugreek seeds (*Trigonella foenum-graecum* L.) and are used in the production of analgesic and anti-inflammatory drugs [Kączkowski 1993]. Fruits and seeds of the carob tree, also called locust beans (*Ceratonia siliqua* L.), are a source of gelling agents used in the food industry. Due to their high viscosity, carob mucilage solutions are used as thickeners of food and emulsions. The pharmaceutical industry uses these compounds to produce formulations for gastrointestinal disorders.

Algal polysaccharides

Polysaccharides can also be extracted from brown algae, whose cells are surrounded by a wall composed of cellulose and pectin layers on the outer and inner surface, respectively. It is estimated that up to 60% of active substances contained in algae are polysaccharide compounds. One of them is hyaluronic acid, which is an

extremely important ingredient in the cosmetic industry. Brown algae also contain large amounts of alginic acid and fucoidan [Matławska 2008, Jękot et al. 2015]. The latter is a polysaccharide composed of α-L-fucopyranose molecules, which can only be connected with each other by 1→3 bonds or alternating 1→3 and 1→4 bonds. Fucoidan also comprises such monosaccharides as galactose, xylose, mannose, glucose, and uronic acid, but their distribution in the polymer is still unknown [Cumashi et al. 2007]. Fucoidan is added to pharmaceutical and cosmetic formulations to extend their shelf life.

Fucoidan is extracted from the extracellular matrix of brown algae *Fucus vesiculosus*, *Fucus evanescens*, *Fucus serratus*, *Fucus distichus*, *Laminaria saccharina*, and other species [Jękot et al. 2015]. These compounds obtained from different algal genera differ in their chemical structure, strength, and activity profile [Hyun et al. 2009]. Currently, two types of fucoidan have been described: F-fucoidan consisting of 95% sulfonated glucose esters and U-fucoidan with 20% of glucuronic acid in its composition. As shown by *in vitro* and *in vivo* animal model studies, fucoidan isolated from different brown algae has no toxic effects even at very high levels of intake. Similarly, clinical studies demonstrated no negative effect of fucoidan on the human organism [Fitton 2011]. In turn, the investigations confirmed the anticancer, anticoagulant, antiviral, anti-inflammatory, and antioxidant effects of this compound.

Polysaccharides from various species of fungi

Various fungal *Basidiomycota* species synthesise metabolites with a broad spectrum of biological activity. Particularly interesting are polysaccharides extracted from fungi, i.e. β-glucans. Polysaccharides exert a multidirectional and wide-range anticancer effect. They limit cell DNA damage, lower the concentration of carcinogens in the human organism, and inhibit their activation and cancer cell development. With their ability to activate the immune system, polysaccharides can support the treatment of cancer and infectious diseases. An important advantage of natural polysaccharides is their ability to abolish the side effects of chemotherapy accompanied by no toxic effects on the human organism [Sułkowska-Ziaja et al. 2005, Turło 2013, Siwulski et al. 2014, Florczak et al. 2016]. Anticancer polysaccharide-based drugs derived from fungi have been applied in treatment of gastrointestinal, breast, cervical, and lung cancers. The following preparations isolated from fruiting bodies, mycelium, or post-culture medium of *Basidiomycota* fungi are examples of such fungal drugs:

Schizophyllan isolated from *Schizophyllum commune* culture medium,
Grifolan isolated from *Grifola frondosa* fruiting bodies and mycelium,
Krestin isolated from *Trametes versicolor* mycelium,
Lentinan isolated from *Lentinula edodes* fruiting bodies.

They were introduced to official therapy by e.g. Japanese researchers [Smith et al. 2002, Turło 2013, Ślusarczyk and Kuraś 2020].

The anticancer properties of fungi are mainly associated with their content of polysaccharide compounds. These organisms are a source of (1→3), (1→6)-β--glucans, (1→3)-α-glucans, and many hetero-β-glucans. The anticancer properties of polysaccharides are determined by their water solubility, molecule size, molecular weight, degree of branching, and occurrence form [Rajewska and Bałasińska 2004]. The highest anticancer activity was determined in the case of high molecular weight β-glucans containing mainly β-(1→3) bonds [Mizuno 1999]. These polysaccharides have been found to exert a cytotoxic effect on cancer cells [Smith et al. 2002].

Special attention has been devoted in recent years to several species of the following medicinal mushrooms due to their content of bioactive compounds.

Hemlock varnish shelf (*Ganoderma lucidum* [W. Curt.: Fr.] P. Karst.) is used in Asian medicine for treatment of liver diseases, hypertonia, arthritis, asthma, and gastric ulcers. This fungus has been cultured for several decades, and is therefore more widely available. It is known as Ling Zhi in China and Reishi in Japan. It is highly valuable due to the high content of polysaccharides. Hot water extracts from the fruiting body contain 51% of polysaccharides and 5% of proteins [Grys et al. 2011]. Over 150 polysaccharides have been isolated from its organs [Yang et al. 2007].

Shiitake mushroom (*Lentinula edodes* [Berk.] Pegl.) biosynthesises many compounds with antibacterial, antiviral, antithrombotic, and anti-hypercholesterolemic activity. Lignins isolated from *L. edodes* cultures are investigated as potential drugs in the treatment of hepatitis B and AIDS. Similarly, the polysaccharides produced by the fungus exert an immunomodulatory effect. They are used in chemoprevention and as supportive cancer therapy to alleviate the undesirable effects of chemotherapy [Turło 2013].

Blazei mushroom (*Agaricus blazei* Murill) contains large amounts of polysaccharides, e.g. β-D-glucan, in addition to vitamins B1, B2, and minerals. Blazei mushroom extracts are very effective in many cancer diseases, as they inhibit the progression of the disease and induce remission. The extracts are particularly helpful in the treatment of liver cancer, cirrhosis, breast cancer, and hepatitis B and C even in cases excluding the application of conventional therapeutic methods such as chemotherapy, radiotherapy, or surgery [Lisiecka et al. 2013].

Wood ear (*Auricularia auricularia-judae*) grows on old or dead wood in forests, parks, or gardens. It usually colonises the elderberry (*Sambucus nigra* L.) but can also be found on other broad-leaved tree species. The polysaccharides contained in its fruiting bodies have immunostimulatory activity. The bioactive compounds produced by the fungus have anti-aggregation activity inhibiting excessive blood coagulation [Yoon et al. 2003]. They also have anti-inflammatory, antibacterial, and hypolipidemic properties [Zeng et al. 2013].

Lion's mane (*Hericium erinaceum* (Bull.: Fr.) Pers.) is a medicinal fungus that has been extensively studied in recent years. The bioactive substances extracted from the fungus exhibit anticancer activity and alleviate the symptoms and effects of Parkinson's and Alzheimer's diseases [Krzyczkowski et al. 2008]. It strengthens the organism, improves digestion, and supports therapy of some cancers [Zhang et al. 2007]. The fungus produces several types of bioactive substances, e.g. intracellular polysaccharides (IPS) and extracellular polysaccharides (EPS), which exert immunostimulatory and anticancer effects [Mizuno et al. 1999, Ślusarczyk et al. 2014]. Due to the legal protection of the species in many countries, including Poland, mycelial cultures besides fruiting bodies can be a source of immunoactive polysaccharides [Malinowska et al. 2009].

Conclusion

Medicinal plants are a source of a huge number of biologically active substances, e.g. polysaccharide compounds, which are currently used in many areas of life, including medicine. Some of them are nutrients and dietary ingredients. They are also used as expectorants and antitussive agents in treatment of respiratory tract inflammation. They are applied in cosmetology due to their protective and softening effect on the skin and mucous membranes. Polysaccharides also exert a positive effect on the immune system. In recent years, polysaccharides with immunoregulatory and anticancer activity have been intensively investigated. Since they are able to activate the immune system, these compounds can support the treatment of cancer. A considerable advantage of natural polysaccharides is their potential to mitigate the side effects of chemotherapy. They also produce no side effects in the human organism. Hence, polysaccharide-containing formulations are becoming increasingly important in the prophylaxis and treatment of many diseases as the main components of novel drugs with high therapeutic potential.

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Polisacharydy roślinne i grzybowe – substancje ważne dla naszego zdrowia i życia

Streszczenie. W pracy przedstawiono charakterystykę najważniejszych polisacharydów roślinnych i grzybowych, takich jak skrobia, celuloza, śluzy roślinne czy wytwarzany przez glony fukoidan. Omówiono ich znaczenie w przyrodzie i w życiu człowieka, a w szczególności wykorzystanie w przemyśle farmaceutycznym i kosmetycznym. Wiele gatunków grzybów również charakteryzuje się zdolnością biosyntezy związków biologicznie czynnych. Produkują one m.in. polisacharydy o aktywności przeciwnowotworowej immunostymulującej. Na przykładzie wybranych gatunków przedstawiono właściwości lecznicze produkowanych przez nie metabolitów wtórnych oraz możliwości ich wykorzystania.

Słowa kluczowe: fukoidan, polisacharydy grzybowe, śluzy roślinne, polisacharydy roślinne

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Health-enhancing properties of lycopene and its use in prevention of lifestyle diseases

Summary. The aim of the study is to present the health-enhancing properties of lycopene and its role in the prophylaxis of lifestyle diseases, i.e. oncological diseases, with particular emphasis on prostate cancer, and cancer diseases affecting pre-menopausal women.

The paper also discusses the preventive effects of lycopene in cardiovascular diseases as well as the potential sources of the compound with antioxidant properties and its use in industry, pharmacy, and cosmetology.

Key words: antioxidant properties, health-enhancing effects, lycopene, plant-origin substances, prophylaxis of lifestyle diseases

Let food be thy medicine and medicine be thy food
Hippocrates

Introduction

Lifestyle diseases, whose development is associated with the degree of public awareness, are the most common cause of mortality worldwide. It has been demonstrated that unhealthy food and lifestyle contribute to the prevalence of 21st century diseases, i.e. ischemic heart disease, atherosclerosis, or cancer. Therefore, it is important to educate society on the importance of consumption of healthy food and its effects on the function of the human organism. Dissemination of the knowledge of healthy and easily accessible natural products is becoming one of the targets of actions for prevention of the spread of many diseases. The prophylaxis of lifestyle diseases includes increased consumption of biologically active compounds, such as lycopene.

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Lycopene – a natural antioxidant

Lycopene is one of the natural antioxidants from the group of carotenoids, i.e. orange-red pigments commonly found in nature [Duliński 2019]. They are characterised by good solubility in fats. They are involved in photosynthesis, during which they protect plants against the adverse effects of radiation, absorb radiation, and convert excess energy into heat [Strzałka and Gabryś 2002, Szterk and Lewicki 2007, Rakowski 2018]. Lycopene is a carotenoid with the highest antioxidant activity. It is a chemical compound with the formula $C_{40}H_{56}$ and molar mass of 536.85 g/mol [Kwiatkowska 2010, Gryszczyńska et al. 2011]. A feature that distinguishes lycopene from other carotenoids is the presence of 11 conjugated bonds, which are involved in elimination of free radicals [Shi and Le Maguer 2000, Belter et al. 2011]. Tomatoes (*Lycopersicon esculentum* L.) are the main source of lycopene, but the compound can also be found in other fruits and vegetables, e.g. papaya, guava, and pink grapefruit. This carotenoid is also found in processed tomatoes, e.g. juices and purees, but its content is relatively low in such products [Wawrzyniak et al. 2015, Górecka et al. 2020]. Tomato processing is associated with the action of high temperature, which damages tomato fruit cell walls, which in turn leads to lycopene extraction [Zalewska-Korona et al. 2013]. Importantly, elevated temperature stimulates lycopene absorption in the human organism with the involvement of the isomerisation process, during which the *trans* lycopene form present in fresh fruit is transformed into the *cis*-isomer [Szajdek and Borowska 2004, Gwóźdź and Gębczyński 2017].

Technological processes applied to tomato fruit yield by-products, e.g. seeds and skins. These wastes are used for extraction of lycopene, which is an extremely valuable ingredient of functional food [Silva et al. 2019]. Food enriched with biologically active substances acquires new sensory and nutritional values [Skiepko et al. 2015]. The conversion of lycopene from the *trans* into *cis* form results in improved solubility of the compound in bile salts. Additionally, the *cis* isomer exhibits higher effectiveness of binding liporoteins and proteins involved in its transport [Skiepko et al. 2015].

The interest in the so-called functional food has contributed significantly to the increased production of foodstuffs containing lycopene in their composition. These include all types of tomato ketchups and concentrates as well as nutrient- and antioxidant-rich dietary supplements and lycopene drinks. They are called anticancer juices due to the high content of lycopene (on average 30 mg in 100 ml of juice). This compound is also widely used in the food industry as a natural pigment for colouring processed food. The presence of lycopene in processed food reduces the content of harmful preservatives such as nitrates(III). The concentration of these compounds in food is reduced by the use of natural additives that have a similar effect to nitrates(III), i.e. they give colour to the products and have anti-antioxidant (preservative) properties. Therefore, as a strong antioxidant and natural dye, lycopene is used in the form of tomato powder for the production

of sausages, as a compound giving the red colouring to these products. The presence of lycopene therefore contributes to reducing the level of harmful preservatives in these products and replacing them with ingredients of natural origin [Skiepko et al. 2015]. The compound is also widely applied in pharmacy and cosmetology. With their valuable biological properties, carotenoids, including lycopene, are frequently consumed by humans as a source of vitamin A. Thanks to their antioxidant abilities, carotenoids form a natural protective barrier against free radicals in the organism. Thus, they play an important role in the fight against the effects of oxidative stress, i.e. one of the causes of the development of lifestyle diseases [Szterk and Lewicki 2007, Wawrzyniak et al. 2015]. As demonstrated by various studies, systematic lycopene supplementation protects the skin immune system and reduces the risk of sunburn. With its antioxidant properties and ability to absorb UV rays, lycopene plays an important role in protection of human skin against the negative effects of UV-A and UV-B radiation; hence, it is widely used in cosmetology and pharmacy [Milani et al. 2017].

The conversion of lycopene from the *trans* into *cis* form during technological processes results in improved solubility of the compound in bile salts. Additionally, the *cis* isomer exhibits higher effectiveness of binding lipoproteins and proteins involved in its transport [Skiepko et al. 2015]. Lycopene is supplied to the human organism with food. It participates in the formation of the lipid phase in the stomach and duodenum. This phase is dispersed in the presence of bile salts and pancreatic lipases. Lycopene is absorbed in the small intestine and colon [Belter et al. 2011]. The absorption has been evidenced to depend on the fat content in the diet. The presence of fats in food increases secretion of bile salts, which enhances lycopene absorption [Kwiatkowska 2010, Wawrzyniak et al. 2015]. The content of lycopene in body tissues is in the range of 0.2–21.4 nmol/g. It is determined not only by the bioavailability of the compound and diet but also by the type of tissue and the ability to eliminate the compound from the organism [Goralczyk and Siler 2004]. The highest lycopene content is observed in testes, adrenal glands, liver, and prostate, whereas its concentration in the brain is substantially lower [Belter et al. 2011].

Health-enhancing activity of lycopene

Lycopene is a carotenoid with strong antioxidant properties. Its antioxidant effect involves elimination of singlet oxygen ($^1O_2$) and free radicals generated in oxidative stress conditions via neutralisation of the reactive species [Przybylska 2020]. This is achieved through radical adhesion, electron transfer, and detachment of hydrogen. Additionally, lycopene enhances the antioxidant response in cells by an impact on their membranes: it increases their flexibility, fluidity, and permeability. Therefore, it protects lipids present in cell membranes against the penetration of free radicals [Belter et al. 2011].

With its properties, lycopene is used both as a component of dietary supplements and as a therapeutic agent. A lycopene-rich diet has a positive effect on health; therefore, it is recommended for prophylaxis of many diseases [Belter et al. 2011]. The compound plays a role in reduction of the incidence of such diseases as prostate cancer, lung cancer, and cervical cancer, as suggested by the results of many epidemiological studies. Lycopene acts as an inhibitor of the development of metastatic foci and proliferation of cancer cells. Due to its ability to lower blood pressure, it is also used to prevent cardiovascular diseases [Gwóźdź and Gębczyński 2017].

Anticancer therapies are frequently based on the health-enhancing activity of plant-derived compounds, such as lycopene, whose anti-carcinogenic potential is associated with antioxidant properties. It is worth noting that lycopene is a compound that can only help reduce the occurrence of cancer, thanks to its preventive antioxidant effects, but it is not widely used in oncological therapies as an anticancer drug [Skiepko et al. 2015]. The positive effect of this carotenoid in cancer prophylaxis is based on its anti-carcinogenic properties. The mechanism of action of lycopene used for the prevention of cancer consists in:

- elimination of substances with carcinogenic potential,
- inhibition of unnatural cell divisions,
- induction of apoptosis, which leads to removal of infected or damaged cells from the organism [Kwiatkowska 2010, Belter et al. 2011].

However, these processes depend on the lycopene concentration in cells and the cell type. *In vitro* studies indicate that this compound induces apoptosis in carcinogenic prostate cells already at a concentration of 10 nM, whereas a potent effect of lycopene is observed at a concentration of 2–4 μM in bowel cancer and Burkitt-type lymphoma cells [Van Breemen et al. 2011]. The anticancer activity of lycopene is associated with cell cycle arrest in the G0-G1 phase [Johary et al. 2012]. The rate of carcinogenic cell divisions and the formation of a malignant tumour are determined by the presence of platelet-derived growth factor PDGF-BB, which is involved in the migration of e.g. melanoma cells. Numerous epidemiological studies indicate that the presence of lycopene in serum has a destructive effect on the growth of these cells and their migration in the organism [Belter et al. 2011, Thies et al. 2017].

Lycopene plays an important role in the prevention of cancer diseases. It is most often used in the treatment of prostate cancer, as it is known to accumulate in prostate tissue in high concentrations [Nordström et al. 2016]. Numerous studies have demonstrated a significant correlation between the consumption of tomato products and delayed carcinogenesis in the prostate gland. It was found that the progression of prostate cancer was inhibited by nearly 30%. This was suggested to be a result of the action of lycopene on androgens, which are involved in the hyperplasia of the gland. In androgen-induced prostate cancer cells, lycopene stimulates the production of special peptides, which significantly reduce the invasiveness of the tumour. These proteins are involved in e.g. reduction of the effects of oxidative stress (ROS) and elimination of free radicals [Goo et al.

2007]. The ability of lycopene to inhibit the proliferation of prostate cancer cells of the LNCaP line (human androgen-sensitive cells) is quite important as well. Such inhibition was observed after administration of successive 15 mM and 25 mM concentrations of lycopene [Yang et al. 2012]. However, prostate cancer can be detected relatively early by measurement of the prostate-specific antigen (PSA). There are, therefore, concerns whether surgical treatment is necessary. For this reason, attention has been focused on the possibility of introducing a non-invasive therapy consisting in the use of preparations that inhibit the development of prostate cancer with no side effects [Sporn and Liby 2013]. It has been revealed that daily consumption of 15–30 mg of lycopene contributes to a significant decrease in the prostate-specific antigen level. The results of research conducted since the 1990s suggest that there is an inversely proportional relationship between the presence of lycopene in the human diet and the development of prostate cancer. The risk of development of prostate cancer in men with daily lycopene intake of 30 mg decreased by nearly more than half compared to those who received lower doses [Zu et al. 2014, Wawrzyniak et al. 2015]. Additionally, regular consumption of lycopene has been found to delay tumour progression, which is reflected in a reduced mortality rate [Zu et al. 2014]. With such biological properties, lycopene is widely used in the prophylaxis of prostate cancer and prostatic hypertrophy [Nelson et al. 2001].

The anticancer properties of lycopene are also useful for prevention of diseases affecting women in the pre-menopausal period, e.g. cervical cancer and breast cancer [Didkowska et al. 2011]. The risk of these diseases in women can be reduced via a decrease in IGF-1 (*insulin-like growth factor*) levels. This is achieved by increasing the production of the insulin-like growth factor-binding protein, which results in a decrease in the level of the factor in blood. The synthesis of the IGF-1-binding peptide can occur only at high lycopene content in serum [Karas et al. 2000, Aguirre et al. 2016]. Lycopene supplementation combined with oncological therapy may accelerate the effects of the treatment, compared with the standard procedure. A diet comprising large amounts of plant-origin products or lycopene supplements is the basis for the prophylaxis of these cancer diseases [Kwiatkowska 2010, Skiepko et al. 2015].

The antioxidant properties of lycopene have been found helpful not only in the prophylaxis of cancer but also in the treatment of cardiovascular diseases, since the compound has the ability to inhibit thrombocyte aggregation and clot formation [Karppi et al. 2013]. One of the main factors inducing the development of cardiovascular disease is atherosclerosis, which involves deposition of cholesterol in the arterial lumen. A continuous process of accumulation of this compound in the blood vessel walls may lead to tissue death due to blockage of the artery lumen by clots impeding free blood flow and oxygenation [Beręsewicz and Skierczyńska 2006, Mozos et al. 2018]. The role of lycopene in the prevention of cardiovascular diseases is associated with its antioxidant capacity as

well as deactivation of cholesterol synthesis [Wawrzyniak et al. 2015]. Inhibition of this process contributes to a decrease in the concentration of this compound in the cell accompanied by an increase in the number of LDL receptors (LDL – a "bad" cholesterol fraction). This results in greater absorption of these substances from plasma [Storniolo et al. 2019]. Clinical studies demonstrated that daily consumption of 60 mg of lycopene for 3 months resulted in a nearly 14% reduction of serum LDL levels [Wiktorowska-Owczarek 2013, Han and Liu 2017].

Conclusion

A proper diet and healthy lifestyle ensure protection of the organism against external factors that may promote the development of lifestyle diseases. Hence, consumption of vegetable products with valuable biological properties becomes extremely important. The presence of lycopene in the human diet is therefore necessary, given the wide range of its health-enhancing effects, i.e. limitation of the development of cancer diseases by e.g. inhibition of the proliferation of carcinogenic cells or activation of apoptosis. Additionally, high serum lycopene concentrations reduce the risk of cardiovascular disease, e.g. atherosclerosis, by inhibition of cholesterol synthesis. Although there are many literature reports and results of numerous studies on the impact of lycopene on the development of 21st century diseases, it is advisable to monitor continuously the health-enhancing effects of the compound on the organism.

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Właściwości prozdrowotne likopenu i jego wykorzystanie w profilaktyce chorób cywilizacyjnych

Streszczenie. Celem pracy jest przedstawienie właściwości prozdrowotnych likopenu oraz jego roli w profilaktyce chorób cywilizacyjnych tj. chorób nowotworowych, ze szczególnym uwzględnieniem nowotworów występujących u mężczyzn, m.in. raka prostaty, oraz nowotworów rozwijających się u kobiet, związanych z wiekiem przedmenopauzalnym. W pracy omówiono także wpływ likopenu na rozwój chorób układu krążenia, potencjalne źródła jego występowania, a także wykorzystanie jego właściwości antyoksydacyjnych w przemyśle, farmacji i kosmetologii.

Słowa kluczowe: właściwości antyoksydacyjne, znaczenie prozdrowotne, likopen, substancje pochodzenia roślinnego, profilaktyka chorób cywilizacyjnych

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Fucoidan – occurrence, activity, and application

Summary. Currently, increasing interest in natural bioactive substances, their activity, and potential practical application in supportive therapy of many diseases or reduction of side effects of conventional treatment has been noted. One of such phytochemicals is fucoidan – a marine polysaccharide consisting of sulphated fucose present in large brown algae. This paper presents the latest available literature data on the characteristics, occurrence, biological activity, and potential uses of fucoidan for improvement of human well-being and health. There are numerous reports in the scientific literature on the multifarious activities of this compound, e.g. anti-inflammatory, antitumor, immunomodulatory, hypolipidemic, antioxidant, anti-complementary, anti-hepatopathy, anti-uropathy, and anti-renalpathy activities as well as gastric protective effects and therapeutic potential in surgery. At present, many investigations are focused on the possible dietary use of fucoidan during chemo- and radiotherapy and its adjuvant effects in cancer treatment.

Key words: algae, biological activity, phytocompound, phytotherapy, polysaccharide

Introduction

Currently, there is a substantial increase in the interest in biologically active chemical compounds derived from natural sources. Special attention is focused on methods for extraction of active substances from marine resources, e.g. algae. They are a valuable source of various groups of active compounds with a broad spectrum of beneficial bioactivity and effects on human health. With their diverse composition, these compounds have been applied in formulations of new natural products in the pharmaceutical, cosmetic, and food industries as well as thalassotherapy and agriculture [Ariede et al. 2017, Hamed et al. 2018, Joshi et al. 2018, Ravishankar and Ambati 2019]. This paper compiles and presents information from

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available literature about the occurrence, properties, and activity of fucoidan, i.e. one of the marine-origin polysaccharides.

Characteristics and occurrence

Fucoidan (IUPAC name) was first discovered and isolated in 1913 from marine brown algae by Professor H.Z. Kylin from Uppsala University in Sweden. Originally named "fucoidin", it is now known as fucan, fucosan, or sulfated fucan [Berteau and Mulloy 2003]. This sulphonated polysaccharide from the group of fucans is present in various species of seaweed, mainly in brown algae, e.g. *Adenocystis utricularis*, *Ascophyllum nodosum*, *Eisenia bicyclis*, *Fucus vesiculosus*, *Macrocystis pyrifera*, *Nemacystus decipiens*, *Saccharina japonica*, *Sargassum fusiforme*, and *Undaria pinnatifida*, in which it accounts for approximately 4% of dry weight. Fucoidan has also been detected in invertebrate marine animals representing echinoderms (e.g. sea urchins – Echinoidea and sea cucumbers – Holothuroidea) [Kannan et al. 2013, Zhao et al. 2018, Mansour et al. 2019].

The structure of fucoidan comprises α-L-fucopyranose molecules linked by 1→3 or 1→3 and 1→4 bonds. Its main chain has α-L-fucopyranose residues, inorganic substituents – sulphate (VI) residues, and organic compounds, e.g. D-glucuronic acid or acetyl residues [Senthilkumar and Kim 2014, Jękot et al. 2015]. Fucoidan is rich in the sulphonated polysaccharide fucose, which is its main sugar component [Mak et al. 2013, Pozharitskaya et al. 2018]. The composition and chemical structure of fucoidan is not constant and depends on the species of the organism used for extraction, which determines its biological and therapeutic properties. There are large differences in its activity, depending on the degree of sulphonation and acetylation, polysaccharide branching, type of bonds, or molecular weight. *Fucus vesiculosus* is the most common of the species used providing the simplest polymer of the whole group with only L-fucose and sulphate units. In other sources, the structure may comprise d-mannose, d-galactose, d-xylose, l-rhamnose, and uronic acid units [Anastyuk et al. 2009, Huang and Lam 2011]. Therefore, there are two types of fucoidan: F-Fucoidan consisting mainly of sulphate L-fucose and U-Fucoidan (uronofucoidan) containing primarily glucuronic acid, i.e. an important substance involved in detoxification of the organism (transformation and removal of xenobiotics). Both these fucoidan forms are applied as nutraceuticals. There are many methods for isolation of fucoidan, including water extraction, acid or alkali extraction, enzyme extraction, and microwave – or ultrasound-assisted extraction.

Fucoidan is mainly contained in the cell wall [Elizondo-Gonzalez et al. 2012]. Its highest concentration has been found in mature sporophylls [Mak et al. 2013]. The compound exhibits a range of diverse biological activity with a wide pharmacological spectrum of antimicrobial, anticancer, anti-inflammatory, anticoagulant, and

antioxidant effects [Lakshmanasenthil et al. 2014, Senthilkumar and Kim 2014, Choi et al. 2015].

Phytotherapeutic activity

Antioxidant activity. Many algal extracts have been analysed in search for pharmacological bioactive compounds with high antioxidant activity [Elizondo--Gonzalez et al. 2012]. Fucoidan extracted from *Ascophyllum nodosum*, *Sargassum binderi*, *S. polycystum*, *S. targassum tenerrimum*, and *Undaria pinnatifida* exhibited a strong dose-dependent antioxidant effect. It has been confirmed as a good source of natural antioxidants [Mak et al. 2013, Lim et al. 2014, Marudhupandi et al. 2014, Yuan and Macquarrie 2015, Palanisamy et al. 2017]. These promising results support the idea of commercialisation of fucoidan as a functional food product or biological ingredient with potent antioxidant properties [Lim et al. 2014]. Investigations conducted by Ajisaka et al. [2016] have evidenced that the antioxidant activity of fucoidans depends on such factors as the number of sulphate groups, position of sulphate groups, type of side chain sugar, linkage of the side chain sugar, and molecular weight.

Antiviral activity. Currently, scientists are searching for new safe antimicrobial substances. Various studies have indicated fucoidan as a compound with strong antiviral activity even at low concentrations (0.001–0.05 μg/ml), regardless of the carbohydrate skeleton. Fucoidan extracted from *Dictyota dichotomia* and *Undaria pinnatifida* exhibited activity against herpes virus type 1 (HSV-1) and type 2 (HSV-2) [Lee et al. 2004, Hayashi et al. 2008, Rabanal et al. 2014]. The compound derived from the former and the latter species was active against Coxsackie virus (CVB3) and human cytomegalovirus (HCMV), respectively [Lee et al. 2004, Rabanal et al. 2014].

In vitro and *in vivo* studies have shown the activity of fucoidan isolated from *Cladosiphon okamuranus* against the bird Newcastle disease (ND) virus in a Vero cell line [Elizondo-Gonzalez et al. 2012]. It has been demonstrated that fucoidan can be useful for the development of pharmacological strategies of treatment and control of Canine Distemper Virus (CDV) infection [Luthuli et al. 2019]. In turn, fucoidan extracted from *Fucus evanescens* has been reported to inhibit mosaic virus infection of tobacco leaves [Lapshina et al. 2006, 2007]. Promising results were also obtained upon application of fucoidan as a new therapeutic drug against Anti-Hepatitis B Virus (HBV). Fucoidan significantly inhibited HBV replication in a mouse model *in vivo* and in HepG2.2.15 cells *in vitro*. Fucoidan from *F. vesiculosus* activates the MAPK-ERK1/2 pathway and subsequently promotes the expression of IFN-α, causing a decrease in the production of HBV DNA and related proteins. Therefore, it may be concluded that fucoidan can be used as an alternative therapeutic strategy against HBV infection. Promising preliminary results of investigations of the therapeutic

potential of fucoidan against HIV have been reported as well. It has been found that fucoidans are able to inhibit HIV-1 infection upon pre-incubation with the virus, but not with cells, and not after infection, showing that they are able to block the early steps of HIV entry into target cells. However, further *in vitro* and *in vivo* studies are necessary before clinical testing [Luthuli et al. 2019].

High molecular weight compounds (1.3-α-1-fucan and galactofucan) isolated from *Saccharina cichorioides* and *S. japonica* have been reported as effective inhibitors of HIV-1 [Prokofjeva et al. 2013]. Similarly, fucoidan extracted from *Sargassum mcclurei*, S. *polycystum*, and *Turbinara ornata* was found to inhibit HIV-1 infection after pre-incubation with the virus by blocking the early stages of HIV entry into target cells. It has been discovered that the structural traits of fucoidans strongly determine their biological activity [Thuy et al. 2015].

Antibacterial activity. Fucoidan fraction-2 (Fu-F2) extracted from *Sargassum polycystum*, which contained 51.12% of total sugar and 20.41% of sulphate, was demonstrated to have significant antibacterial activity of against *Pseudomonas aeruginosa* (MTCC No: 2642), *Streptococcus mutans* (MTCC No:896), *Escherichia coli* (MTCC No: 40), and *Staphylococcus aureus* (MTCC No: 96) strains obtained from the Microbial Type Culture Collection and Gene Bank (MTCC), Institute of Microbial Technology, IMTECH, Chandigarh, India. The minimum inhibitory concentrations (MIC) of Fu-F2 against these strains were estimated by the authors at 50, 100, 200, and 200 μg/ml, respectively. In turn, the minimum bactericidal concentration (MBC) was 200 μg/ml against *P. aeruginosa* and 300 μg/ml against the other strains. Therefore, these findings revealed the highest antibacterial activity of Fu-F2 against *P. aeruginosa* [Palanisamy et. al. 2019].

In contrast to unprocessed fucoidan administered at 10 mg/ml, depolymerised fucoidan isolated from *Laminaria japonica* was found to inhibit effectively the proliferation of *E. coli* ATCC25922 and *S. aureus* ATCC29213 strains obtained from the American Type Culture Collection (Manassas, Virginia, USA). The depolymerised fucoidans were more effective against *E. coli* than against *S. aureus*. The minimum inhibitory concentrations obtained for five depolymerised fucoidan fractions with different molecular weight regions were 6.25 (F1, <6 kDa) and 8 mg/ml (F2, 6–20 kDa; F3, 20–50 kDa; F4, 50–80 kDa; F5, >80 kDa) for the former pathogen. The following values were reported in the case of the latter pathogen: 8.5 (F1, <6 kDa), 12.5 (F2, 6 kDa), and 10 mg/ml (F3, 20–50 kDa; F4, 50–80 kDa; F5, >80 kDa). The MBC of fucoidan fractions against *E. coli* reported by the authors was 10 (fraction F1), 12.5 (fractions F2, F3, F4), and 25 mg/ml (fraction F5). Values of 12.5 (F1) and 25 mg/ml (F2-F5) were shown in the case of *S. aureus*. The MIC and MBC values for the unprocessed fucoidan (no depolymerisation) were 25 mg/ml. Thus, lower molecular weight and stronger polyanionic properties have been reported to increase antibacterial activity. The bactericidal activity of fucoidan is associated with impairment of membrane integrity and

change in membrane fluidity [Liu et al. 2017]. Crude fucoidan from *Sargassum polycystum* was shown to inhibit the growth of *Escherichia coli*, *Staphylococcus aureus*, and *Vibrio harveyi* with MIC values of 6, 12, and 12 mg/ml, respectively, and the observed inhibition zone was 10 mm [Chotigeat et al. 2004].

Similarly, fucoidan isolated from *Sargassum wightii* exerted an antibacterial effect against eight human pathogens. The inhibitory activity of fucoidan (500 μg/ml) against the growth of bacterial strains obtained from the Rajah Muthiah Medical College, Annamalai University, Tamil Nadu, India was as follows (mm): *Vibrio cholerae* 18.6 ±0.32 > *Pseudomonas aeruginosa* 16.23 > *Shigella sonnie* 14.83 > *Klebsiella* sp. 14.3 > *Proteus proteus* 13.2 > *Escherichia coli* 11.03 > *Klebsiella pneumoniae* 9.6 > *Salmonella typhi* 8.6. In turn, the following values (mm) were shown in the positive control (500 μg/ml of tetracycline): *Klebsiella* sp. 27, *P. proteus* 26, *E. coli* 24, *V. cholerae* 22, *S. typhi* 21, *P. aeruginosa* 20, *S. sonnie* 20, and *K. pneumoniae* 19. The MIC value, i.e. the lowest fucoidan concentration that completely inhibited the visible growth of the tested microorganisms was (μg/ml) as follows: 31.35 (*V. cholerae*), 62.5 (*P. aeruginosa*), 125 (*E. coli, Klebsiella* sp., *P. Proteus*, and *S. sonnie*), and *250* (*K. pneumoniae* and *S. typhi*). The minimum bactericidal concentration (MBC) of fucoidan reached the lowest value of 62.5 μg/ml against *V. cholerae*, 150 μg/ml against *P. aeruginosa*, and 250 μg/ml against *E. coli, Klebsiella* sp., *P. proteus*, and *S. sonnie*. In turn, the highest value of this parameter (500 μg/ml) was found in the activity against *K pneumoniae* and *S. typhi* [Marudhupandi and Kumar 2013].

Fucoidan- and berberine-containing nanoparticles were demonstrated to be active against *S. aureus* and *E. coli*, with slow release of berberine for 24 h inhibiting bacterial growth [Yu et al. 2015]. Choi et al. [2015] revealed that fucoidan exerted synergistic effects when administered with oxacillin or ampicillin as well as an antimicrobial effect with resistance regulation against clinically isolated methicillin-resistant *Staphylococcus aureus* (MRSA). The values of the minimum inhibitory concentrations (MICs)/ minimum bactericidal concentrations (MBCs) for fucoidan against all the tested bacteria strains ranged between 64–512/256–2048 μg/ml vs. 32–1024/64–1024 μg/ml for ampicillin and 8–64/16–256 μg/ml for oxacillin, respectively. Furthermore, the MIC and MBC values were reduced to one of half eighth in the combined use of fucoidan with antibiotics. Treatment with 1/2 MIC of fucoidan with 1/2 MIC of the antibiotics for 2–6 h resulted in an higher increase in the bactericidal rate expressed in CFU/ml units than that observed in the separate application [Choi et al. 2015].

Thus, it has been postulated that fucoidan can be used as a natural antibacterial agent against multidrug resistant bacteria and a natural and safe antibiotic [Marudhupandi and Kumar 2013, Choi et al. 2015].

Anticoagulant activity. Fucoidan extracted from *Fucus evanescens* exhibited *in vitro* and *in vivo* heparin-like anticoagulant activity, i.e. inhibition of thrombin via plasma antithrombin III [Kuznetsova et al. 2003]. Mansour et al. [2019]

revealed high anticoagulant potential of fucoidan from the sea cucumber *Holothuria polii*. This effect was mediated essentially by heparin cofactor II and to a lesser extent by antithrombin. It was also attributed to the high sulphate content and the abundance of disulphated fucose residues in the *H. polii* fucoidan. As suggested by Wang et al. [2011], the anti-coagulant activity of fucoidan is dependent on its molecular weight, sulphate group/ total sugar ratio, sulphate position, sulphate degree, and glycoside branching.

Conclusion

Given its multifarious biological activity, fucoidan isolated from different species has been extensively investigated with the focus on its beneficial bioactivities in a range of human health conditions. Promising findings have been reported showing the potential application of fucoidan in adjunctive therapy for the conventional treatment of many different diseases with few side effects. Fucoidan, which is a compound with high activity and a wide spectrum of effects, can be regarded as a potential biocompound in the phytotherapy of some diseases and as an agent in thalassotherapy, i.e. treatment with the use of sea-related factors, including algae, seaweed, and various sea-derived substances. The actions and bioactivities of fucoidan are dependent on the algal species from which they are isolated, molecular weight, and methods of extraction. Generally, low molecular weight fucoidan possesses greater bioactivities than fucoidan with high molecular weight. The compound may play an important role in further development of new effective pharmaceuticals and cosmeceuticals. There is a need for further detailed studies elucidating the mechanisms of action and metabolic pathways of fucoidan, including its pharmacological interactions and safety. Exploration of the chemistry and structural variability of each type of fucoidan is highly important as well.

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Fukoidan – występowanie, działanie i zastosowanie

Streszczenie. Obecnie znacząco zwiększa się zainteresowanie biologicznie aktywnymi substancjami pochodzenia naturalnego, ich działaniem oraz możliwościami praktycznego wykorzystania w terapii wspomagającej wielu jednostek chorobowych lub ograniczeniu występowania skutków ubocznych leczenia konwencjonalnego. Jednym z wielu fitozwiązków jest fucoidan, polisacharyd z alg morskich składający się z usiarczanowanej fruktozy, występujący u brunatnic. W niniejszej pracy, na podstawie dostępnych danych literaturowych, zaprezentowano wybrane najnowsze informacje odnośnie charakterystyki, występowania, biologicznej aktywności oraz potencjalnych możliwości zastosowania fucoidanu w poprawie samopoczucia i zdrowia ludzkiego. W literaturze naukowej znane są liczne doniesienia na temat różnej aktywności tego związku, w tym działania przeciwzapalnego, przeciwnowotworowego i immunomodulującego, efektu obniżenia poziomu lipidów we krwi, właściwości przeciwutleniających i antykomplementarnych, aktywności antyhepatopatycznej, antyuropatycznej i antynefropatycznej, a także ochronnego wpływu na żołądek oraz terapeutycznego potencjału w chirurgii. Obecnie wiele badań koncentruje się na możliwości zastosowania fukoidanu w diecie w trakcie chemio- i radioterapii oraz na jego wspomagającym wpływie w leczeniu nowotworów.

Słowa kluczowe: glony, biologiczna aktywność, fitozwiązek, fitoterapia, polisacharydy

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Bromelain – antibacterial activity and application in dermatology and cosmetology

Summary. *Ananas comosus* is the primary source of bromelain, which is a mixture of proteolytic enzymes influencing a number of metabolic pathways. Due to its proteolytic activity, it is used in many various branches of industry, e.g. pharmaceutical and cosmetic industries. This review, based on original research reports, presents the biochemical characteristics and the current knowledge of the antibacterial activity and application of bromelain in dermatology and cosmetology together with information on its safety and contraindications to the use of the phytocompound.

Key words: *Ananas comosus*, phytotheraphy, phytocompounds, pineapple, proteolytic enzymes

Introduction

Bromelain is a crude protein extract from various organs (stems, leaves, fruits) and parts of the infructescence (crown, peel, core) of some species of the genus *Ananas* from the family *Bromeliaceae* [Bresolin et al. 2013, Ali et al. 2015, Vicente et al. 2016, Das and Bhattacharyya 2017, Kahiro et al. 2017, Hatano et al. 2018, Kargutkar and Brijesh 2018, Saptarini et al. 2019]. The best-known source of bromelain is the pineapple (*Ananas comosus* (Linn.) Merr), which is indigenous to South America (south-eastern Brazil, Argentina, and Uruguay) and is cultivated in Central America (Hawaii, Costa Rica) as well as Asian countries, including the Philippines, Thailand, Indonesia, Malaysia, India, and China [Crestani et al. 2010, Arshad et al. 2014, Kusumaningtyas et al. 2015]. The species was introduced in Europe by Christopher Columbus, who brought the *piña de Indes*, i.e. 'the pine of the Indians' plant from Guadeloupe to Spain in 1493 [Carlier et al. 2007].

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Primarily, the pineapple stem is used for production of bromelain for commercial use, probably because its usable quantities are readily extractable after fruit harvest. Additionally, mature stems have a substantially higher concentration of bromelain than fruits, from which it was first isolated [Ketnawa et al. 2012, Ramli et al. 2017, 2018]. Due to its proteolytic activity, bromelain is used in various branches of industry: textile, chemical, medical, pharmaceutical, and cosmetic industries as well as food industry to manufacture bakery, dairy, and brewery products [Polaina and MacCabe 2007, Spir et al. 2015, Amini et al. 2016, Lourenço et al. 2016, Ramli et al. 2017, Singh et al. 2018]. Bromelain facilitates digestion of proteins through partial hydrolysis of molecules into smaller peptides, thereby increasing their availability in food [Fennema 1996]. It is also used as a nutraceutical [Esam 2020]. Proteinases contained in bromelain are efficient phytotherapeutic agents [Pillai et al. 2013, Silva 2016, Matschke et al. 2017, Mohamad et al. 2019]. Bromelain has been used for many years in folk medicine for various health problems. Already in ancient times, native peoples of Central and South America applied pineapple dressings to wounds and skin injuries to reduce inflammation and drank pineapple fruit juice to alleviate stomach ache and indigestion. The therapeutic properties were ascribed to bromelain used as a pharmaceutical product in 1957, when it was first applied as a therapeutic agent blocking pro-inflammatory metabolites [Amini et al. 2016]. At present, bromelain is used in phytotherapy as an anti-inflammatory [Bernela et al. 2016, Kargutkar and Brijesh 2018, Sharma and Sharma 2018], antibacterial [Praveen et al. 2014, Ataide et al. 2017, Loon et al. 2018], antithrombotic [Arenajo et al. 2018], and anti-oxidative [López-Pedrouso et al. 2020] agent.

The aim of the review was to present the current data from original research reports on the therapeutic effects and application of bromelain in dermatology and cosmetology with indication of contradictions to its use.

History of discovery

Bromelain was first identified and isolated in 1891 by a Venezuelan chemist Vicente Marcano in the process of fermentation of pineapple fruits, i.e. a plant with a long history of use in *traditional folk* medicine among the natives of South and Central Americas. However, Marcano died before he had the results of his last investigations published. In 1892, the isolation and characterisation of the compound discovered by Marcano were completed by an American physiological chemist Russell Henry Chittenden in collaboration with Elliott P. Joslin and Frank Sherman Meara. The compound was described as "proteolytic ferment of pineapple juice" and initially referred to as "bromelin"; later, the name was changed into "Bromelain" [Chittenden et al. 1892]. Further intensive research was conducted in the early 1950's at the Pineapple Research Institute in Hawaii, where a large number of *A. comosus* and other species of the family *Bromeliaceae* were

studied. Additionally, a similar proteolytic formulation was produced on a pilot scale. Since then, the term "bromelain", which was originally given to the protease mixture of stem and fruit pineapple juice, was used as the collective name of enzymes found in any member of the family *Bromeliaceae*. Since the report by Heinicke and Gortner [1957] showing that the mature pineapple stem is a rich source of an enzyme mixture similar to bromelin identified by Marcano, the "Stem Bromelain", commonly referred to as "Bromelain" has been produced on a commercial scale and used in pharmaceutical preparation.

Physical properties and biochemical characteristic

Bromelain is a mixture of proteolytic enzymes, i.e. thiol endopeptidases, and non-protease components. It is a light brown solid with a characteristic intense aroma. The freezing and boiling points of the substance are –7.2°C and 58.9°C, respectively [Smith-Marshall and Golden 2012, Novaes et al. 2014]. Proteases, which are the main component of bromelain, include 'stem bromelain' (EC 3.4.22.32; 80%), i.e. a major peptidase present in the stem, 'fruit bromelain' (EC 3.4.22.31; 5%), which is a major enzyme fraction found in the fruit juice, and two minor cysteine endopeptidases i.e. ananain (EC 3.4.22.31; 5%) and comosain [Polaina and MacCabe 2007, Nadzirah et al. 2013, Amini et al. 2016, Ramli et al. 2018].

The stem bromelain is a glycosylated single-chain protein belonging to the papain family with molecular weight 26–37 kDa, isoelectric point (pI) of 9.55, and diffusion coefficient of 7.77×10^{-7} cm^2/s. It contains 212 amino acid residues, including seven cysteines, one of which is involved in catalysis and the other six are associated in pairs forming three disulphide bridges [Soares et al. 2012]. This type of bromelain is mainly produced and widely available on the market, as its extraction is relatively inexpensive and the stem is a waste product [Muntari et al. 2012, Bala et al. 2013]. For a majority of substrates, the optimum pH for bromelain activity is in the range of 6–8.5, and the optimum temperature range is 40–60°C. However, this enzyme is usually unstable and sensitive in stress conditions and its function may be decreased in the presence of organic solvents and chemicals [Soares et al. 2012, Coelho et al. 2014, Martins et al. 2014a, 2014b, Manzoor et al. 2016]. Cysteine is a much more efficient activator of bromelain than other thiols. The stem bromelain shows strong preference for benzyloxycarbonyl--Arg-7-amino-4--methyl-coumarin among small-molecule substrates. It is very slowly inactivated by E-64, i.e. an epoxide that can irreversibly inhibit a wide range of cysteine peptidases [Polaina and MacCabe 2007, Amini et al. 2016].

Stem and fruit bromelains are immunologically distinct [Rowan and Buttle 1994, Barrett et al. 2004]. The fruit bromelain is a single-chain glycosylated protein with molecular weight in the range of 24.5–32 kDa and pI value of 4.6 [Nadzirah et al. 2013]. It is characterised by a considerably higher proteolytic

activity and broader specificity than the stem bromelain, but the latter compound exhibits high proteolytic activity against protein substrates with a preference for polar amino acids in positions P_1 and P_1'. In contrast to the stem bromelain, the fruit-derived compound is rarely used due to its limited availability and high production costs [Polaina and MacCabe 2007, Corso et al. 2012, Amini et al. 2016, Bernela et al. 2016]. The non-protein components of bromelain include escharase, which contributes to debriding effects, phosphatases, glucosidases, peroxidases, cellulases, glycoproteins, carbohydrates, and several protease inhibitors [Polaina and MacCabe 2007, Silvestre et al. 2012, Smith-Marshall and Golden 2012, Amini et al. 2016, Ramli et al. 2018].

Antibacterial activity

Literature data indicate the potential of application of bromelain as an antibacterial agent against potent aerobic and anaerobic periodontal pathogens [Praveen et al. 2014]. Aerobic strains *Enterococcus fecalis* (ATCC No. 35550) and *Streptococcus mutans* (ATCC No. 25175) showed sensitivity (minimum inhibitory concentration MIC) at the lowest concentration of 31.25 and 2 mg/mL, respectively, while the MIC value for isolated strains of anaerobic organisms *Porphyromonas gingivalis* (ATCC No. 33277) and *Aggregatibacter actinomycetemcomitans* (ATCC No. 29523) was estimated at 4.15 and 16.6 mg/mL. The authors hypothesize that the antibacterial action of bromelain is related to prevention of bacterial attachment. It is likely that bromelain exerts effects against intestinal pathogens (*Escherichia coli*, *Enterococcus fecalis*) via interaction with intestinal secretory signalling pathways, which includes the adenosine 3':5'-cyclic monophosphatase, guanosine 3':5'-cyclic monophosphatase, and calcium-dependent signalling cascades. Moreover, bromelain also has anti-adhesive properties, which prevent bacteria from adhering to specific glycoprotein receptors located on the intestinal mucosa.

As suggested by Ali et al. [2015], crude fruit bromelain extract (1.8 mg/mL) shows more potency in inhibiting Gram-positive than Gram-negative bacteria isolated from fresh and overnight meat. Antibacterial activity was estimated at different temperatures (25, 37, and 45°C) in neutral pH media and at different pH values (7 and 10) at a temperature of 37°C. It was found that crude bromelain was effective as an antimicrobial agent against *E. coli* and *Proteus* spp. The growth inhibition zone observed at 25, 37, and 45°C at pH 7.0 was 19.00, 22.23, and 24.00 mm, respectively, for *E. coli* and 12.67, 14.67, and 13.67 mm for *Proteus* spp. In turn, at alkaline reaction (pH 10) and a temperature of 37°C, the *E. coli* and *Proteus* spp. inhibition zone was 8.33 and 18.67 mm, respectively. Another (specific) strain of Gram-negative *E. coli* as well as two Gram-positive *Streptococcus pyogenes* and *Bacillus subtilis* strains were resistant to crude bromelain at all experimental temperature values and neutral pH. Nevertheless, complete growth inhibition of the microorganisms was observed at a temperature of 37°C

and alkaline reaction (pH 10). Gram-positive *Corynebacterium* spp. exhibited the lowest inhibition of all the tested microorganisms, with an 8.33 mm inhibition zone at 37°C and 45°C and 9.33 mm at 25°C. Moreover, bromelain failed to inhibit the growth of this strain at pH 10. A combination of crude bromelain and an antibiotic (Ampicloxacillin 2.5mg/mL+ crude extract 0.9mg/mL) had a more potent effect than either the standard or crude bromelain. The proposed mechanism by which bromelain inhibits the growth of bacteria consists in hydrolysis of some peptide bonds present in the bacterial cell wall, and the higher resistance of Gram-positive than Gram-negative bacteria to bromelain is associated with the differences in the structure of their cell walls.

The results of investigations conducted by Ataide et al. [2017] on Gram-negative *Escherichia coli* (ATCC 25922) and *Pseudomonas aeuroginosa* (ATCC 9721) strains and a Gram-positive *Staphylococcus aureus* (ATCC 10390) strain indicated a different direction than that shown by Ali et al. [2015], who found that crude bromelain was more effective in inhibiting Gram-positive than Gram-negative bacterial growth. To evaluate the antimicrobial activity, Ataide et al. [2017] examined the following solutions: (i) initial bromelain solution; (ii) residual bromelain solution, and (iii) bromelain solution after release from bacterial nanocellulose membranes. They found that bromelain inhibited the growth of all tested microorganisms, especially the post-release solution. Additionally, the antimicrobial activity of bromelain was associated with its enzymatic activity. The minimum inhibitory concentration (MIC) value of the bromelain post-release solution was 0.99 U/mL, 0.25 U/mL, and 0.5 U/mL for *E. coli*, *P. aeruginosa*, and *S. aureus*, respectively, and was 6, 6, and 12 times higher, respectively, than the MIC of the initial solution. It may be concluded that bromelain itself has antimicrobial and antioxidant activities; however, after incorporation into bacterial nanocellulose membranes, its antimicrobial activity substantially increases. Furthermore, the mucoadhesive properties of bacterial nanocellulose membranes decreases after bromelain loading, which can be explained by the presence of H-bonds between bromelain and bacterial nanocellulose membrane, leading to a decrease in molecular interactions between bacterial nanocellulose and mucin.

The study conducted by Anjos et al. [2016] showed an antibacterial effect of bromelain against *Alicyclobacillus acidoterrestris* with minimum inhibitory and bactericidal concentrations of 62.5 and 250 μg/mL, respectively. The bromelain concentration of 4 × MIC was sufficient to eliminate 4 logs of the micro-organism after 24 h of incubation. Based on results of studies conducted with the use of enzyme inhibitors specific for cysteine proteases, the authors claim that the antibacterial activity of bromelain is probably not related to its proteolytic activity, but other activities, such as amidase and esterase activities. As suggested by Loon and coworkers [2018], the antibacterial effect of pineapple extracts on *Staphylococcus*

aureus is related to the bromelain compound and its phytochemical factor such as Vitamin C and flavonoid with a MIC range of 1.56–0.78%.

As revealed by Hidayat et al. [2018], crude extract of core bromelain shows antibacterial activity against *Staphylococcus epidermidis, Propionibacterium acnes, Staphylococcus aureus*, and *Escherichia coli* with an inhibition zone of 8.5, 9.5, 11, and 11 mm, respectively. The antibacterial potency of ammonium sulphate and acetone fractionated bromelain was only seen in *P. acnes* and *S. aureus*. This is an advantage of bromelain as an antibacterial agent, as *S. epidermidis* and *E. coli* are part of normal bacterial flora in the organism. Of all fractions analyzed, the highest specific activity and purity was observed in the case of the ammonium sulphate fraction with a saturation level of 20–50% (specific activity 70 U/mg, 5.3–fold higher purity level compared to its crude extract) and the acetone fraction with a 50–80% saturation level (specific activity 19.736 U/mg, 2.5–fold higher purity level compared to its crude extract). Therefore, the fractionation with the use of ammonium sulphate was better to yield higher specific activity than in the case of acetone. It turn, bromelain fractionated with acetone showed better antibacterial activity than in the case of ammonium sulphate, with a 6 mm and 12.5 mm inhibition zone for 20–80% ammonium sulphate and 50–80% acetone, respectively. Therefore, pineapple core bromelain crude extract and its fractions may be an efficient antibacterial agent against *P. acnes* and *S. aureus*. However, further investigations focused on determination of the minimum inhibitory concentrations are needed in order to specify the effective dose of bromelain use.

In spite of the strong evidence that bromelain is an agent with a potential broad application as an antibacterial and anti-inflammatory drug in medicine and dentistry, its mechanisms of action need to be elucidated in detailed studies.

Application in dermatology

Reports from 1964 [Pierce 1964] indicated efficiency of bromelain treatment in a 32-year-old patient with scleroderma, i.e. an autoimmune disease of the connective tissue characterized by fibrosis and thickening of various tissues. Three-month bromelain therapy resulted in considerable long-term (over a year) improvement of patient's health status and resolution of clinical symptoms, including the hide-bound appearance of the skin of the face and upper extremities and patchy depigmentation of the forehead and scalp. In turn, Massimiliano et al. [2007] found high effectiveness of bromelain in the treatment of patients with pityriasis lichenoides chronica – a rare cutaneous disorder of unknown aetiology characterized by the development of multiple, scaly, erythematous to brown papules on the trunk and extremities. In patients suffering from the disease administered bromelain orally for three months (40 mg 3 times a day for 1 month, 40 mg twice a day for 1 month, and 40 mg/day for 1 month) exhibited complete resolution of the disease symptoms and no side effects of the therapy. This effect is attributed to the

antiviral, antibacterial, anti-inflammatory, and immunomodulatory properties of bromelain.

Bresolin et al. [2014] reported promising results facilitating consideration of incorporation of the enzyme bromelain from pineapple peel into dermatological bases for potential therapeutic application. They found that when the enzyme was incorporated into the Lanette cream and lotion as well as Carbopol gel and Chemyunion® cream and lotion at a concentration of 0.5% (w/w), these bases were stable when kept in a refrigerator at 4°C. In such conditions, their organoleptic properties (appearance, colour, smell, and sensitivity to touch) and activity were well preserved. Bromelain is recommended as an ingredient in therapeutic products with local soothing effects in e.g. contact dermatitis, insect bites, psoriasis, seborrhoea, eczema, abrasions, and skin cuts and burns [Richon 2017]. It is an ingredient in some dermatological formulations. Bromelain-containing formulations (lotions, creams, and ointments) as topical therapeutic agents for the safe and efficient treatment of rosacea, eczema, and skin pruritus have been developed [Richon 2017, 2018a, 2018b, 2020]. Bromelain is also used in skin care to reduce petechiae, oedema, and adverse effects of cosmetic, laser, and surgical skin treatments. It has been approved by the German Commission E for postsurgical and/or posttraumatic oedema, particularly of the nasal and paranasal sinuses characteristic of some plastic surgery. A patented cutaneous tape containing bromelain is also available in Europe for debriding scar tissue [Ho et al. 2016]. Bromelain is also applied to reduce inflammation and support wound healing [Maurer and Eschmann 2010]. Oral administration of 500 mg/2x/day/year exerts a positive effect on persistent erythema caused by recurrent herpes simplex infections [Aktaş 2019].

Belcher et al. [2016] obtained promising results in terms of the effectiveness of bromelain in the proteolysis of bullous pemphigoid antigen 2 (BPAG2), also known as type XVII collagen or BP180. The pathogenic anti-BPAG2 antibodies present in the serum of patients with the severe blistering bullous pemphigoid disease recognize and preferentially bind the non-collagenous 16A domain of collagen XVII present in hemidesmosomes causing the dermal-epidermal separation in neonatal and adult mice as well as in cryosections of human skin. Anti-BPAG2 autoantibodies are detected not only in patients with bullous pemphigoid but also in those with pemphigoid gestationis, cicatricial pemphigoid, and linear IgA dermatosis.

Application in cosmetology

Bromelain is a valuable ingredient for *cosmetic* formulations. It is used in face-care products to provide gentle peeling effects [Polaina and MacCabe 2007]. Pineapple extract has an exfoliating, peeling, cleansing, anti-inflammatory, brightening, moisturising, and anti-cellulite effect. It also reduces blemishes and swelling, improves skin tone, and brightens skin discolorations. It soothes

acne, irritated dry, couperose, sensitive, and retinoid-treated skin and reduces proneness to seborrhoea. It is an ingredient of brightening and peeling cosmetics designed for acne, seborrhoeic, dry, sensitive, and poorly moisturised skin. Due to its physicochemical form and intended use, the recommended concentrations of bromelain extract in different cosmetics may vary. Since bromelain may cause irritation and sensitisation, its formulations should contain recommended concentrations for face masks (up to 2%), facial cream, tonic, and serum (up to 0.5%), and hair products (up to 1%) [Polaina and MacCabe 2007, Levy and Emer 2012].

Cream containing encapsulated bromelain heals and prevents ingrown hairs. The cream basically comprises a carrier, preferably water, and an active amount of encapsulated bromelain preferably comprising inert silicone, especially cyclomethicone. Its composition enriched by addition of beeswax, sunflower oil, shea butter, or tea tree oil is recommended in the treatment of acne [Borden and Artriss 2013]. In terms of the stability of bromelain extract in anhydrous gel, cream, and cream-gel, the latter proved to be the most suitable of the three cosmetic bases for transferring bromelain. The optimal storage temperature was 4°C [Spir et al. 2015].

Precautions and contraindications

Folk medicine and modern clinical studies indicate very low toxicity and absence of side effects of bromelain. Although the use of bromelain is safe, some caution should be exercised [Braun et al. 2005, Barrera-Núñez et al. 2014, Sabhaz et al. 2015, Ho et al. 2016]. The compound may interact with some anticoagulants and sedatives and increase the concentration of antibiotics (amoxicillin) in blood, thereby increasing their activity, which may pose some risk in certain cases. Diabetic patients should use bromelain with caution due to its high glycaemic index. Bromelain seldom causes side effects other than occasional mild gastrointestinal distress or allergic reactions. Nevertheless, allergy sufferers, especially those allergic to carrots, celery, fennel, grass pollen, and wheat, may develop cross-reactivity to bromelain. The safety of the substance for young children, pregnant or nursing women, or those with liver or kidney disease has not been established. Unripe pineapple and juice from its leaves can speed up labour or cause a miscarriage. Pineapples should not be consumed by patients with gastric and duodenal ulcers. Since bromelain might retard blood clothing, it should not be combined with anticoagulant/antiplatelet drugs such as aspirin, clopidogrel, diclofenac, ibuprofen, naproxen, dalteparin, enoxaparin, heparin, indomethacin, ticlopidine, and warfarin without medical supervision [Heck et al. 2000, Maurer 2001, Shahid et al. 2002, Johann et al. 2011].

Conclusion

With its effective antibacterial effect, bromelain can be used in dermatology and cosmetology. It can be an effective antibacterial agent against periodontal pathogens (*Enterococcus fecalis*, *Streptococcus mutans*, *Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans*) and other pathogenic strains (*Alicyclobacillus acidoterrestris*, *E. coli*, *P. acnes*, *S. aureus*, *Proteus* spp.). When released from nanocellulose membranes, bromelain inhibits the growth of *Escherichia coli*, *Pseudomonas aeuroginosa*, and *Staphylococcus aureus*. Despite the strong evidence that bromelain can be potentially broadly applied as an antibacterial drug in medicine and dentistry, further detailed investigations are required to elucidate the mechanisms of its action, with a focus on the differences in the sensitivity between Gram-negative and Gram-positive bacteria. Proteinases contained in bromelain are effective therapeutic agents in the treatment of skin lesions, e.g. erythema, dermatitis, psoriasis, rosacea, eczema, and postoperative ecchymoses. Bromelain has been shown to bring therapeutic effects in patients with pityriasis lichenoides chronica. Additionally, bromelain is a component of cosmetics for the care of acne, seborrhoeic, dry, and sensitive skin. An additional advantage of bromelain is its safe use, i.e. low toxicity and no side effects. Therefore, this phytocompound has been approved for phytotherapeutic applications.

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Bromelaina – działanie antybakteryjne oraz zastosowanie w dermatologii i kosmetologii

Streszczenie. Podstawowym źródłem bromelainy jest *Ananas comosus.* Jest ona mieszaniną enzymów proteolitycznych oddziaływujących na szereg szlaków metabolicznych. Ze względu na aktywność proteolityczną bromelię wykorzystano w wielu różnych gałęziach przemysłu, w tym farmaceutycznym i kosmetycznym. W niniejszej pracy, opierając się na oryginalnych doniesieniach naukowych, zaprezentowano charakterystykę biochemiczną i aktualny stan wiedzy dotyczący aktywności antybakteryjnej oraz zastosowania bromelainy w dermatologii i kosmetologii wraz z informacjami o jej bezpieczeństwie i przeciwwskazaniach do stosowania.

Słowa kluczowe: *Ananas comosus*, fitoterapia, fotozwiązki, ananas jadalny, enzymy proteolityczne

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Antimicrobial activity of *Hedera helix* L.

Summary. The raw material *Hederae folium* provides bio-compounds used in the pharmaceutical industry. At present, new sources of antimicrobial compounds should be isolated and identified, given the frequently inappropriate use of antibiotics, serious adverse events, and development of antibiotic resistance. The present paper, based on original research reports, compiles information and the current state of the knowledge of the antibacterial, antiviral, antifungal, and leishmanicidial activity of *Hedera helix* L. leaf extracts with a focus on biologically active substances contained therein.

Key words: antibacterial activities, antifungal activities, antiviral activities, ivy, *Leishmania*, phytocompounds, phytotherapy, antiparasitic effect

Introduction

The common ivy *Hedera helix* L. is widespread in the natural environment in Poland. It is grown and recommended for planting as an ornamental species. Ivy leaves (*Hederae folium*) are the raw material of this species. The raw material is a rich source of bioactive compounds, mainly triterpene saponins. This group of compounds exhibits a number of health-enhancing activities, e.g. mucolytic, spasmolytic, anti-inflammatory, antiviral [Lutsenko et al. 2010, Hong et al. 2015, Wang et al. 2015], antibacterial [Uddin et al. 2011, Pop et al. 2017, Akhtar et al. 2019], antifungal [Favel et al. 1994, Moulin-Traffort et al. 1998, Mel'nichenko et al. 2003, Prescott et al. 2014, Parvu et al. 2015, Roşca-Casian et al. 2017], and antihelminthic effects [Cioaca et al. 1978, Delmas et al. 2000, Lutsenko et al. 2010].

The therapeutic activity of *H. helix* has been used e.g. in pharmaceutical industry. The Polish pharmaceutical market offers over-the-counter formulations containing triterpene saponins. These include syrups, tablets, oral drops, soft

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tablets, and oral fluids. Some of them are used for treatment of symptoms of acute upper respiratory tract infections [Kemmerich et al. 2006].

In the current search for natural phytocompounds with potential application in medicine, *H. helix* L. is one of the investigated plant species. This need to find and identify new sources of antimicrobial substances is associated with e.g. the frequent inappropriate use of *antibiotics*, serious adverse events, and the development of *antibiotic resistance* [Kemmerich et al. 2006, Simoes et al. 2009]. Animal studies and clinical trials have confirmed the antimicrobial efficacy of biologically active substances isolated from *H. helix* L. [Hong et al. 2015, Saleem et al. 2019, Mama et al. 2020, Manderson 2020].

The aim of the study was to present the antiviral, antibacterial, antifungal, and leishmanicidial activity based on the latest literature reports on the antimicrobial effects of biologically active substances extracted from *Hedera helix* L. leaves. The literature was searched in various online resources in the Pub Med, BioMed Central, and Google Scholar databases available at the authors' universities.

Antibacterial activity

Extracts from *H. helix* herb contain various classes of secondary metabolites with strong antibacterial properties. Chloroform and methanolic extract were found to contain alkaloids and saponins, whereas tannins were identified in ethyl acetate and methanolic extracts; additionally, all these extracts contained terpenoids. Infrared analyses of the various types of ivy extract revealed the presence of N-O, C=O, C-H, C=C, N-H, NO_2, and C-O-C bond stretching. Ethanolic and methanolic extracts exhibited significant antibacterial activity against *Bacillus subtilis* with an inhibition zone of 18 mm and against *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Escherichia coli* with an inhibition zone of 10 mm. However, no activity against *Klebsiella pneumoniae* was detected. The hexane extract was active against *S. aureus*, *K. pneumoniae* and *B. subtilis* with an inhibition zone between 10 and 16 mm, but not against *E. coli* and *S. epidermidis*, while the chloroform extract was active only against *B. subtilis* with an inhibition zone of 16 mm [Uddin et al. 2011].

Pop et al. [2017] analysed ethanolic extract from different ivy organs, i.e. leaves, flowers, and mature and immature fruits, to detect antibacterial activity against Gram-negative (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella typhimurium* ATCC 14028) and Gram-positive (*Bacillus cereus* ATCC 11778, *Listeria monocytogenes* ATCC 19114, *Staphylococcus aureus* ATCC 49444) strains. *S. aureus* was recognised as the most sensitive species to both immature fruit and flower extracts, with values of 0.078 mg/mL and 0.15 mg/mL, respectively and MBC values of 0.15 mg/mL MIC and 0.3 mg/mL, respectively. These two extracts showed good antibacterial capacity against *L. monocytogenes* with the identical MIC value of 0.15 mg/mL and the same

MBC value of 0.3 mg/mL. In comparison, the MIC value for streptomycin against *S. aureus* and *L. monocytogenes* was 0.03 and 0.015, respectively. Extracts from all the other plant organs tested exerted a low growth inhibitory effect on all experimental bacterial strains, with a MIC value of 0.5 mg/mL, which is the upper measurable limit in assessment of the effectiveness of antibacterial activity.

In search for natural bioactive substances with high bactericidal activity that could be used in practical human and veterinary medicine, a potent antibacterial effect on ethanolic *H. helix* leaf extracts was determined against *Campylobacter jejuni* iATCC11322, *Listeria monocytogenes* ATCC 19112, *Proteus vulgaris* ATCC 13315, *Rhodococcus equi* ATCC 6939, *Staphylococcus epidermidis* ATCC 25923, and *Staphylococcus aureus* ATCC14990. The width of the growth inhibition zone in the case of these bacterial strains was 12.4, 9.3, 10.8, 11.8, 26.3, and 23.5 mm, respectively [Zazharskiy et al. 2020].

Promising results were obtained regarding the possibility of application of *H. helix* leaf extracts for the biosynthesis and enhancement of the effectiveness of the antimicrobial properties of silver nanoparticles (AgNP). Silver nanoparticles synthesised with the use of ivy extracts showed markedly higher antimicrobial activity against Gram-positive *B. subtilis* ATCC 1715 and Gram-negative *K. pneumoniae* ATCC 1290 strains in comparison to both $AgNO_3$ and raw plant extracts. Both these strains were characterised by similar sensitivity to silver nanoparticles and a similar growth inhibition zone under the influence of silver nitrate [Abbasifar et al. 2017].

Investigations conducted on mice demonstrated an anti-inflammatory effect of Hederacoside-C (HDC), i.e. one of the compounds contained in *H. helix* leaf extracts, on *Staphylococcus aureus* – induced acute lung inflammation mediated via toll-like receptors (TLRs) and their downstream signalling pathway *in vivo* and *in vitro*. Animals treated with HDC exhibited markedly reduced pulmonary oedema, white blood cell counts, wet-dry ratio (W/D), and myeloperoxidase (MPO) activity. This bioactive phytocompound attenuated the levels of expression of mitogen-activated protein kinases MAPKs, including p38, ERK, JNK, and the nuclear factor kappa-light-chain-enhancer of activated B cells NF-κB, including the p65 and IκB-α pathways. Moreover, it suppressed the expression of TLR2 & TLR4 proteins, downregulated the gene expression of pro-inflammatory cytokines (IL-6, IL-1β, and TNF-α), and upregulated the gene expression of anti-inflammatory cytokine IL-10 [Akhtar et al. 2019].

Antifungal activity

The cytotoxic saponin α-hederin contained in *H. helix* leaves exhibited antifungal activity against *Candida albicans* at a minimum inhibitory concentration of 25 μg/mL. Observations conducted using a transmission electron microscope showed that α-hederin induced changes in the ultrastructure of this fungus, i.e.

modifications of cellular contents and alterations of the cell envelope with degradation and death of the yeasts. These changes were accompanied by disturbances in the function of membranes, in particular the plasmalemma [Moulin-Traffort et al. 1998]. Although the cytotoxicity of saponins is often attributed to membrane damage, Prescott et al. [2014] reported that α-hederin did not induce hypersensitivity with an aminophospholipid translocase deletion in a *Saccharomyces cerevisiae* strain that is frequently hypersensitive to membrane damaging agents. As suggested by these researchers, the haploinsufficiency profile of a-hederin was most similar to that reported for caspofungin, which inhibits the synthesis of the fungal cell wall.

The results reported by Parvu et al. [2015] indicate high antifungal activity of ethanolic extracts from fresh ivy flowers and fruits against plant pathogenic fungi. In this case, the flower extract was more potent than the fruit extract. The flower extract inhibited germination and growth of *Aspergillus niger* with a MIC of 12%, *Botrytis cinerea* and *Sclerotinia sclerotiorum* with a MIC of 8%, *Fusarium oxysporum* f. sp. tulipae, and *Penicillium gladioli* with MIC 10%. In turn, the MIC of the fruit extract was 14% for *A. niger*, 10% for *B. cinerea* and *S. sclerotiorum*, and 12% for *F. oxysporum* f. sp. *tulipae* and for *P. gladioli*. In comparison, the MIC value of the antimycotic drug fluconazole was 30% for *A. niger* and *P. gladioli*, 12% for *B. cinerea*, 10% for *F. oxysporum* f. sp. tulipae, and 8% for *S. sclerotiorum*. However, the authors did not assess the activity of single constituents of the extracts. Nevertheless, based on the identification and quantitative analysis of phenolic compounds, they assumed that the antifungal properties of the analysed extracts were associated with the content of p-coumaric acid, kaempferol, and rutoside, and the mechanism of their action is related to enzyme inhibition by the oxidized compounds, possibly through reaction with sulfhydryl groups or nonspecific interactions with proteins.

Roşca-Casian et al. [2017] assessed the *in vitro* antifungal activity of ethanolic *H. helix* leaf extracts against phytopathogenic fungi causing some of the most frequent diseases of vegetables and ornamental plants. The ivy leaf extract inhibited the germination and growth of fungi causing grey mould (*Botrytis cinerea*, *B. tulipae*), blue mould (*Penicillium gladioli*), and white rot (*Sclerotinia sclerotiorum*) of plants, with a MIC of 10%. A less potent inhibitory effect was shown in the case of *Fusarium oxysporum* f. sp. *tulipae*, with a MIC of 12%. In turn, *Aspergillus niger*, with a MIC of 14%, was the most resistant of all the studied fungi. The MIC values of the synthetic antifungal drug fluconazole reported by the authors in the case of these fungi were in the range of 8–30%. Therefore, the ivy leaf extract was regarded as a strong and cost-effective agent against fungal diseases in plants. Based on the analysis of the chemical composition of the ivy leaf extract, the authors attributed its antifungal activity to polyphenols (rutin, quercetin, kaempferol) and sterols (stigmasterol), which were abundant in the extract, and to saponins. Concurrently, they underline the

need for further research focused on assessment of the antifungal activity of each extract component.

Antiviral activity

Hand, Foot, and Mouth Syndrome (HFMS) is a common paediatric infectious disease. The symptoms of HFMS include fever and exenthema localised on mucous membranes as well as palmar and plantar surfaces. These symptoms may be accompanied by pharyngitis. This infection most often affects children under the age of 5–10 [Zhang et al. 2011, Wang et al. 2015]. HMFS is a viral infection caused mainly by human enterovirus 71 (EV-A71) and *Coxsackie virus* (CV-A16) [Messacar et al. 2018, Guerra et al. 2020].

Hederasaponin B exhibits antiviral activity against sub-genotypes C_3 and C_4 of enterovirus 71 (EV71). Song et al. [2014] conducted a study on the Vero cell line to assess the antiviral activity of hederasaponin B and ethanolic *H. helix* L. extract containing this compound. In both these cases, they evidenced a cytopathic effect as well as inhibition of the synthesis of the EV-A71 viral capsid protein by hederasaponin B.

In turn, Hong et al. [2015] documented that ivy extract administered orally to mice enhanced the antiviral effect of suboptimal oseltamivir doses against influenza A/PR/8 (PR8). Hedrasaponin F, i.e. one of the components of *H. Helix* L. leaf extract, significantly reduced the cytopathic effect on PR8-infected A549 cells in infected mice. The antiviral activity of suboptimal oseltamivir doses did not provide effective protection against PR8 infection, whereas oral administration of ivy extract as part of therapy increased the antiviral efficacy of oseltamivir, which mitigated the inflammation in the airways by lowering the concentration of proinflammatory cytokines. Effective activity of hederacoside C applied at a concentration of 100 μg/ml against another influenza virus (A2/Japan-305) was demonstrated as well [Lutsenko et al. 2010].

Leishmanicidial activity

Leishmaniasis is a tropical disease caused by protozoa of the genus *Leishmania*. These parasites are transmitted to humans and animals by a species-specific vector, i.e. mosquitoes [Reithinger et al. 2007, Pace 2014]. *Leishmania* spp. attack white blood cells (macrophages, granulocytes), which is followed by penetration of internal organs by the protozoa and immune disorders [Naderer and McConville 2007]. The disease is manifested as skin, mucocutaneous, and the most severe systemic form (visceral leishmaniasis, also known as kala-azar) [Salman et al. 1999, Postigo 2010, Basmaciyan and Casanova 2019].

Antileishmanial activity of saponins contained in ivy leaf extracts against the promastigote and amastigote forms of *Leishmania infatum* (isolated from a dog) and *L. tropica* (isolated from a human) was demonstrated in *in vitro* studies. The efficacy of monodesmosides (α-, β-, and γ-hederin, hederagenin) against promastigote forms was comparable to that of pentamidine used in treatment of protozoal infections, including leishmaniasis. In the case of the amastigote forms, only hederagenin exhibited potent activity comparable to that of N-methylglucamine antimonate, which is a drug applied in leishmaniasis therapy. An *in vitro* study showed leishmanicidic activity of *H. helix* saponins, which was similar to that of pentamidine administered in the treatment of parasitic infections or lung infections caused by the promastigote forms of *L. infantum* and *L. tropica*. In turn, hederagenin exhibited high activity against amastigote forms, and this effect was equivalent to that found for N-methylglucamine antimonate [Majester-Savornin et al. 1991].

In vivo investigations on Balb/c mice did not confirm the leishmanicidial effect of *H. helix* extracts. In comparison with the placebo control and negative control groups, there was no reduction of the size of the major skin lesions and no complete resolution of minor lesions caused by *Leishmania major* (MHOM/64/IR/ER75) in the experimental groups treated with 20 and 70% alcoholic ivy extract. Although a decrease in the amastigote counts of the skin lesions was found in the placebo group and in the group receiving the 20% ivy extract after the therapy period, the number of parasites in the negative control as well as in the 70% ivy extract group was not reduced [Hooshyar et al. 2014]. Given these discrepant results, further investigations of the anitileishmanial effect of the main components in *H. helix*, especially hederasaponin (saponin K10), should be carried out.

Conclusion

The literature data presented in this paper indicate the high antimicrobial effectiveness of alcoholic *H. helix* extracts with emphasis on the role of saponins (α-hederin, hederasaponin), polyphenols (rutin, quercetin, kaempferol), and sterols (stigmasterol) as the main biologically active compounds present in these extracts. It is presumed that the extracts from various ivy organs (leaves, flowers, fruits) may be important in practical and veterinary medicine, as they exhibit antibacterial activity against Gram-positive bacteria (*Bacillus* spp., *Listeria monocytogenes*, *Rhodococcus equi*, *Staphylococcus* spp.) and Gram-negative bacteria (*Campylobacter jejuni*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*) as well as antiviral (*human enterovirus 71*, *Coxsackievirus CV-A16*, influenza A/PR/8 virus, and A2/Japan-305 influenza virus), antifungal (*Candida albicans*), and antiparasitic (*Leishmania* spp.) effects. The promising results of the scientific reports presented in this

paper suggest that ivy extracts may be a potential therapeutic agent with comparable efficacy to drugs used in conventional therapy against many bacterial (acute lung inflammation, listeriosis, salmonellosis), viral (HFMS, influenza), fungal (candidiasis), and parasitic (leishmaniasis) diseases. Moreover, fresh ivy flowers and fruits were recognized as an effective factor in the biocontrol of plant fungal pathogens, such as: *Aspergillus niger*, *Botrytis cinerea*, *Fusarium oxysporum* f. sp. *tulipae*, *Penicillium gladioli*, *Sclerotinia sclerotiorum*. However, further research is necessary to identify biologically active compounds with antimicrobial activity in ivy extracts and to elucidate the molecular mechanisms of the action of both raw extracts and their components.

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Przeciwdrobnoustrojowa aktywność *Hedera helix* L.

Streszczenie. Surowiec *Hederae folium* dostarcza biologicznie czynne związki chemicznych stosowane w przemyśle farmaceutycznym. Obecnie, biorąc pod uwagę często niewłaściwe stosowanie antybiotyków, poważne działanie niepożądane i rozwój oporności na antybiotyki, należy wyizolować i zidentyfikować nowe źródła związków przeciwdrobnoustrojowych. W niniejszej pracy, bazując na oryginalnych doniesieniach naukowych, zebrano informacje i zaprezentowano aktualny stan wiedzy na temat antybakteryjnej, antywirusowej, przeciwgrzybicznej oraz skierowanej przeciw *Leishmania* spp. przeciwpasożytniczej aktywności ekstraktów z liści *Hedera helix* L. z uwzględnieniem występujących w nich substancji biologicznie czynnych.

Słowa kluczowe: działanie antybakteryjne, działanie przeciwgrzybicze, działanie antywirusowe, bliszcz pospolity, *Leishmania*, fitozwiązki, fitoterapia, działanie przeciwpasożytnicze

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Spasmolytic, expectorant, and anti-inflammatory activity of *Hedera helix* L. saponins

Summary. The biologically active compounds in *Hedera helix* leaves are represented by e.g. triterpenoid saponins: α and β-hederin and hederacoside B and C. With their biological activity, these phytocompounds are used in the pharmaceutical industry. The present paper describes the spasmolytic, expectorant, and anti-inflammatory effects of saponins and the mechanisms of their action based on a review of original research literature.

Key words: common ivy, phytocompounds, supportive therapy, saponins, *Hederae folium*

Introduction

Researchers are currently searching for phytochemicals with various pharmacological effects. Progress in various fields of science, e.g. biochemistry, genetics, and molecular biology, has contributed to determination of the structure of phytocompounds and their effect on the organism. Bioactive chemical compounds are used as ingredients of medical products applied in supportive therapy of many diseases [Trute et al. 1997, Mendel et al. 2011, Uddin et al. 2011, Pizzorno et al. 2016, Bezruk et al. 2020a, 2020b]. One of the many medicinal plant species, the common ivy (*Hedera helix* L.), is a source of bioactive substances with health-enhancing properties [Roşca-Casian et al. 2017, Shawky and El Sohafy 2020].

The bioactive components contained in *H. helix* L. leaves are represented mainly by triterpenoid saponins of the oleanan type accounting for approximately 5% of dry weight (d.w.) [Sieben et al. 2009, Marczyński et al. 2011]. This group of compounds comprises derivatives of hederagenin aglycones [Gaillard et al. 2003, Tatia et al. 2019], oleanolic acid aglycones [Lutsenko et al. 2010, Pop et al. 2017], and their glycosides: monodesmosides (α-hederin and β-hederin) [Demirci

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et al. 2004, Guelcin et al. 2004, Sieben et al. 2009] and bidesmosides (hederacoside B and C) [Lutsenko et al. 2010, Kim et al. 2013, Hussien and Awad 2014].

A key role in the total biological activity of saponins is played by α-hederin and hederacoside B and C [Wolf et al. 2009, Hussien and Awad 2014, Yu et al. 2016, Kim et al. 2017, Sun et al. 2017, Shawky and El Sohafy 2020]. The following phenolic compounds have been identified as well rutin, kaempferol 3-O-rutinoside, quercetin 3-O-glucoside, kaempferol 3-O-glucoside, quercetin, kaempferol, chlorogenic acid, neochlorogenic acid, *3,5*-d*icaffeoylquinic acid*, rosmarinic acid, caffeic, protocatechuic acid [Trute and Nahrstedt 1997, Roşca-Casian et al. 2017, Bezruk et al. 2020b, Shawky and El Sohafy 2020], a coumarin compound scopolin [Moshaie-Nezhad et al. 2019], and sterols: stigmasterol, campesterol, and spinasterol [Lutsenko et al. 2010, Pop et al. 2017, Roşca-Casian et al. 2017].

Among these bioactive phytocompounds, mainly saponins are used in the pharmaceutical industry due to their secretolytic [Wolf et al. 2009], spasmolytic [Trute et al. 1997, Sieben et al. 2009, Mendel et al. 2011, 2012, Greunke et al. 2015], bronchodilatory [Cianchino et al. 2007, Lang et al. 2015, Rehman et al. 2017] and anti-inflammatory [Gepdiremen et al. 2005, Dumitriu et al. 2013, Rai 2013, Hussien and Awad 2014, Lutsenko et al. 2017] effects.

The aim of the study was to present the current knowledge of the spasmolytic, anti-inflammatory, and expectorant activity saponin compounds contained in *Hedera helix* L. leaves based on scientific literature data.

Characterisation of *Hedera helix* L.

The common ivy (*Hedera helix* L.) from the family Araliaceae is native to Asia Minor and the Caucasus. It is an evergreen climber with creeping or erect woody shoots (woody chameophyte) growing up to 30 m in height. The single most often 5-lobed leaves are composed of a different-length petiole and leathery, shiny leaf blades; they are arranged alternately on the stem. *H. helix* L. grows all over Europe. The species was introduced to North America, where it is currently considered an invasive plant. In Poland, it occurs in the natural environment throughout the country, except for the northeast. It is a medicinal, poisonous, and protected plant [Czerpak and Jabłońska-Trupuć 2008, Witkowska-Żuk 2013, Rutkowski 2019].

H. helix L. is a traditional medicinal plant in Europe. Its leaves are the herbal raw material included in pharmacopoeias (*Hederae folium*). It is a source of bioactive compounds with important biological activities [Hussien and Awad 2014].

Spasmolytic activity substances

The spasmolytic activity of extracts and/or substances isolated from ivy, e.g. α-hederin, has been documented in several *in vitro* and *in vivo* studies. Trute et al. [1997] reported that the spasmolytic activity of ivy leaf extracts was determined mainly saponins, due to their relatively high concentration, and to a lesser extent by other compounds such as dicaffeoylquinic acids and flavonol derivatives. The main antispasmodic compounds were quantified in six different ivy dry extracts, in which the concentration of hederacoside C and α-hederin was in the range of 5.0–11% and 1.2–2.5%, respectively, and the hederagenin content did not exceed 0.01%. Based on the papaverine equivalent value PE (activity of 1 g of the tested substance to the activity of *x* mg of papaverine), potent activity against acetylcholine-induced contractions of an isolated guinea-pig ileum was found for α-hederin and hederagenin (PE = 55 and 49), phenolic compounds: quercetin and kaempferol (PE = 54 and 143), and 3,5-dicaffeoylquinic acid (PE = 22). In turn, the PE value for hederacoside C was approx. 6.

Results of a respiration study indicate that α-hederin found in blood plasma at a concentration of 0.66 μM was sufficiently bioavailable to exert a β-mimetic and spasmolytic effect. Studies of the effect of α-hederin derived from a dry *H. helix* L. leaf extract on the behaviour, dynamics, and regulation of β2-adrenergic receptors have demonstrated that the spasmolytic and secretolytic activity of hederin is determined by the accumulation of β-receptors. It was found that α-hederin (0.5 and 1 μM) inhibited the terbutaline-stimulated internalization of the β2-AR in alveolar epithelial type II cell line (A549). It was also found during preincubation of stably transfected human embryonic kidney 293 cells (HEK293) with 1 μM α-hederin that the compound inhibited internalization of β2-AR-GFP fusion proteins and increased the intracellular cAMP level after stimulation with 1 μM terbutaline. In turn, the hederacoside C and hederagenin saponins did not influence either the binding behaviour of the β2-AR or the intracellular cAMP level [Sieben et al. 2009].

Mendel et al. [2011, 2012] revealed that α-hederin (25–320 μM) significantly changed the spontaneous motor activity of rat stomach smooth muscle. Additionally, hederacoside C applied at a concentration of 350 μM and the total dry *H. helix* L. extract at a dose containing 60 μM of hederacoside C induced remarkable contraction of smooth muscles. The authors assume that the cholinergic pathways do not participate in the α-hederin-evoked contraction of rat isolated fundus and corpus stomach strips.

Greunke et al. [2015] conducted a systematic study on the influence of the main ingredients of dry ivy leaf extracts on the β2-adrenergic responsiveness of human airway smooth muscle cells (HASM cells). They identified β-hederin as another component that was presumably responsible for the β2-mimetic effects. Of all the substances tested (protocatechuic acid, neochlorogenic acid, chlorogenic acid, cryptochlorogenic acid, rutin, kaempferol-3-O-rutinoside, 3,4-, 3,5-,

and 4,5-dicaffeoylquinic acid, hederacoside B, and β-hederin), β-hederin was found to inhibit the internalization of GFP-tagged β2AR in stably transfected HEK293 cells. HASM cells pretreated with β-hederin (1 μM, 24 h) showed a significant increase in β2AR binding. A significant increase was also shown in the cAMP formation in the case of β-hederin-pretreated HASM cells after stimulation with 10 μM of terbutaline and simultaneous stimulation with 10 muM terbutaline and 10 μM forskolin.

Expectorant activity

The expectorant activity of alcohol extracts from *H. helix* was applied in the 19th century in traditional herbal medicine for the treatment of respiratory problems [Stauss-Grabo et al. 2011, Gul et al. 2017]. The expectorant effect of ivy extracts is associated with their content of bioactive chemical compounds from the group of triterpenoid saponins hederacoside (B, C, F, G, H, and F) and α-hederin (monodesmoside). The compounds irritate mucous membranes, which leads to increased secretion of mucous and ensures expectorant effects [Sieben et al. 2009, Cwientzek et al. 2011]. Extracted ivy saponins are used as an expectorant to cure acute catarrh and in treatment of fungal infections of the upper respiratory tract [Gul et al. 2018].

It is believed that the expectorant activity of saponins is mediated by the gastric mucosa, with reflex stimulation of the bronchial mucous glands via the parasympathetic pathway [Wolf et al. 2009]. It has been found that α-hederin is an agonist of β_2-adrenergic receptors in the lungs and bronchi, which indicates that it has spasmolytic activity similar to that of conventional drugs against asthma [Schulte-Michels et al. 2016]. In turn, hederacoside C and hederagenin did not affect the binding behaviour, dynamics, and regulation of these receptors [Sieben et al. 2009, Wolf et al. 2009].

In terms of the healing effect on respiratory infections, it has been reported that hederacoside C is converted into α-hederin by esterases and its glucone hederagenin acts as a G protein-coupled beta-2 adrenergic receptor in lung cells and exerts a beta-2 sympathomimetic effect. It is also used topically to treat cellulite with success [Hegener et al. 2004]. Since a majority of literature reports are focused on the expectorant effect of *H. helix* extracts and there are sparse data on the expectorant activity of their components, i.e. saponins, there is a need for further investigations in this field to elucidate the mechanisms of action of these compounds.

Anti-inflammatory activity

Saponins exhibit varied levels of anti-inflammatory activity, which is associated with their chemical structure [Gepdiremen et al. 2005, Sylla et al. 2019]. As shown in a study conducted by Süleyman et al. [2003], crude and purified saponin *Hedera helix* extracts were effective inhibitors of carrageenan- and cotton-pellet-induced acute and chronic inflammation in rats. Purified saponin extracts at a dose of 100 mg/kg body wt. exerted a substantially stronger chronic anti-inflammatory effect than the same dose of crude saponin extract, with 60% and 49% efficacy, respectively. In turn, crude saponin extract at 50, 100, and 200 mg/kg body wt. doses with an anti-inflammatory effect of 51, 77, and 77 %, respectively, was more potent than purified one with efficacy of 42, 60, and 68% in alleviation of acute anti-inflammation. However, although they had high efficacy, both these extracts exerted a weaker anti-inflammatory effect than indomethacin, which is a nonsteroidal anti-inflammatory drug commonly used as a prescription medication to reduce inflammation-induced fever, pain, stiffness, and swelling. As suggested by the author, the mechanism of the anti-inflammatory effect of ivy saponin extracts may depend on inhibition of the formation of some inflammation mediators [Süleyman et al. 2003].

It has been suggested that *H. helix* triterpene saponins are more potent in the inhibition of the release of histamine and/or serotonin than prostaglandin and/or bradykinin. Therefore, these compounds are more effective in the first than second phase of acute inflammation. According to the cotton pellet-induced chronic inflammation model, saponins from crude and purified ivy extracts may exert their effects by inhibiting the functions of macrophages and fibrosis [Kulkarni et al. 1986, Ionac et al. 1996, Süleyman et al. 2003].

Similar investigations were conducted by Gepdiremen et al. [2005], who assessed the anti-inflammatory potential of α-hederin (monodesmoside) and hederasaponin-C isolated from *H. helix* given orally at concentrations of 0.02 and 20 mg/kg body wt. in carrageenan-induced acute paw oedema in rats, which closely resembles human arthritis. Both these saponins were found to be ineffective in the first phase of acute inflammation. Hederasaponin-C, in contrast to α-hederin, exerted a highly satisfactory anti-inflammatory effect in the second phase, although its activity was not as strong as that of indomethacin. As suggested by the authors, hederasaponin-C may exert its anti-inflammatory effects by blocking bradykinin or other inflammation mediators. The latter effect may occur via prostaglandin pathways. Regarding the structure-activity relationship, it is likely that sugars in position C3 and the Rha7-Glc1-6Glc moiety in position C28 are essential for the acute anti-inflammatory effect.

As indicated by literature data, due to the high concentration of hederacoside C, 70% ethanol *H. helix* leaf extract has not only anti-inflammatory but also bactericidal activity and can successfully be used for production of effective mucolytic preparations for the treatment of acute and chronic inflammation-associated

bronchopulmonary infections. A reduction of dystrophy, a correction of hemodynamic disorders, and a decrease in the reactivity of bronchus-associated lymphoid tissue have been shown. The pharmacological effects were associated with the increased exudation with the release of fluid and erythrocytes from the microvasculature. The inhibition of reactions in hyperplastic lymphoid tissue may be an indirect effect [Lutsenko et al. 2017].

Dumitriu et al. [2013] assessed the vascular anti-inflammatory effects of bidesmosidic saponins (10, 15, and 20 μM) from vegetal raw material (*H. helix* leaves). They found that, during stimulation with TNF-α for acute phase systemic inflammation, ivy saponins had a concerted effect inhibiting secretion of pro-inflammatory cytokines IL6 and IL8 cytokines and expression of intracellular and vascular molecules (ICAM and VCAM) that mediate adhesion and transendothelial migration of leucocytes at the inflammation site and the vascular endothelial growth factor (VEGF) in a dose-dependent manner. Among the tested saponin doses, the most active dose on IL6 and IL8 inhibition was 20μM, acting on both cytokines and inducing a double effect: arrest of the progression of acute inflammation (IL6 pathway) and inhibition of neutrophil activation (IL8 signalling). These researchers demonstrated that *H. helix* saponins inhibited the inflammation triggered by bacterial stimuli (lipopolysaccharide) by blocking the secretion of the IL6 and IL8 cytokines and the pro-angiogenic factor VEGF, which involves halting the extracellular pro-inflammatory signalling cascade and reducing vascular permeability. In this unspecific type of inflammation, ivy bidesmosidic saponins did not affect significantly the expression of adhesion molecules (ICAM and CAM). Therefore, in response to the stimulation with LPS, all the saponin doses tested in the experiment (10–20 μM) acted as an anti-inflammatory drug, inhibiting neutrophil recruitment and activation (IL8 inhibition) and avoiding the chronic stage of inflammation (IL6 inhibition). The dose of 15 μM had the most potent activity and strengthened the anti-inflammatory action through the VEGF inhibition effect on vascular permeability, reducing the electrolyte transfer at the endothelial level and minimizing oedema.

The mechanisms of the anti-inflammatory effects of *H. helix* saponins are not fully elucidated; hence, there is a need for further comprehensive investigations in this field.

Conclusions

Triterpene saponins, mainly α- and β-hederin and hederacoside B and C, are the dominant group of bioactive chemical compounds contained in *H. helix*. The literature reports presented in this paper have provided promising data on the effective spasmolytic, expectorant, and anti-inflammatory activity of these compounds. It is believed that the spasmolytic and secretolytic hederin activity is determined by the accumulation of β-receptors. The expectorant activity of

saponins is thought to be mediated by the gastric mucosa, with reflex stimulation of the bronchial mucous glands via the parasympathetic pathway. The mechanism of the anti-inflammatory effect of ivy saponin extracts may depend on inhibition of the formation of some inflammation mediators. It is assumed that triterpene saponins are more potent in blocking the release of histamine and/or serotonin than prostaglandin and/or bradykinin. However, there are also data indicating that hederasaponin-C may exert its anti-inflammatory effects mainly by blocking bradykinin. In the case of LPS- and TNF-α-induced inflammation, bidesmosidic ivy saponins blocked the secretion of IL6 and IL8 cytokines and the pro-angiogenic factor VEGF, which involves blockage of the extracellular pro-inflammatory signalling cascade and reduction of vascular permeability. During the stimulation with TNF-α, but not in the case of LPS-stimulation, saponins significantly influenced the expression of adhesion molecules (ICAM and CAM). Further detailed investigations are necessary to identify and elucidate the mechanisms of action and safety of use of triterpene saponins exhibiting spasmolytic, expectorant, and anti-inflammatory activity.

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Działanie spazmolityczne, wykrztuśne i przeciwzapalne saponin *Hedera helix* L.

Streszczenie. Wśród biologicznie aktywnych związków chemicznych w liściach *Hedera helix* są m.in. saponiny triterpenoidowe: α i β-hederyna, hederakozyd B i C. Z uwagi na swoją aktywność fitozwiązki te mają szerokie zastosowanie w przemyśle farmaceutycznym. W niniejszej pracy zaprezentowano działanie spazmolityczne, wykrztuśne i przeciwzapalne saponin, uwzględniając mechanizmy ich działania, na podstawie aktualnego stanu wiedzy oraz przeglądu oryginalnej literatury naukowej.

Słowa kluczowe: bluszcz pospolity, fitozwiązki, leczenie wspomagające, saponiny, *Hederae folium*

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Phytotherapy of rhinitis and rhinosinusitis – overview of recent research

Summary. Rhinitis and rhinosinusitis are the most common upper respiratory tract diseases. Despite their mostly viral aetiology, they are often treated with antibiotics. Clear guidelines on herbal symptomatic therapy may improve the outcome of therapies and reduce the risk of adverse effects.

The aim of this study was to present available studies on the phytotherapy of rhinitis and rhinosinusitis (allergic and infectious).

There are not many clinical studies on human models, but the available ones suggest a possibility of satisfactory effectiveness of herbal therapies. More future investigations are needed to obtain information on the effective doses, indications, and possible adverse effects of herbal substances.

Key words: allergic rhinitis, rhinosinusitis, medicinal herbs

Introduction

Rhinitis and rhinosinusitis are one of the most common causes of appointments with general practitioners or otolaryngologists. Depending on the duration of symptoms, these diseases are divided into acute and chronic. The etiopathology of these conditions can be infectious, allergic, or posttraumatic [WHO 2019, Foden et al. 2013].

The risk factors for the whole population include exposure to smoke, level of air pollution, and contact with infected people, especially in small spaces. The diseases have most commonly a viral aetiology (up to 90%). This means that antibiotics should be avoided to prevent development of antibiotic resistance. Nevertheless, most (60 to 80%) upper respiratory tract infections are

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treated with antibiotics, while symptomatic treatment should be prescribed in the first step when there is no suspicion of a bacterial aetiology. This suggests using intranasal steroids and mucocutaneous decongestants or herbal products [Pietruszewska et al. 2018].

According to the WHO definition, allergic rhinitis, or hay fever, and allergic sinusitis are reactions of the organism to exposure to allergens. Blockade of sinuses can be caused by the common cold, allergic rhinitis, or nasal polyps. Based on the intensity of symptoms, it can be mild or moderate/severe [WHO 2019].

Chronic rhinosinusitis is a process that lasts at least 12 weeks. It is mostly treated with endoscopy/surgery (anatomical abnormalities) or antibiotic therapy. Ciprofloxacin or amoxicillin with clavulanic acid have clinical cure and bacterial eradication rates up to 91%. Functional endoscopic sinus surgery has a success rate of 73–97.5%, but up to 15% of surgeries require revision. Persistent inflammation may be responsible for recurrent chronic sinusitis, when the total immunoglobulin IgE level does not vary substantially one year after the surgical procedure [Hatipoğlu and Rubinstein 2008].

According to the PROSINUS study, there may be a significant overuse of diagnostic tools and medications for viral and post-viral rhinosinusitis [Jaume et al. 2018]. As shown by the Clinical Practice Guideline from 2015, there are no recommendations regarding the use of herbal therapy for allergic rhinitis [Seidman et al. 2015]. A guide to the management of acute rhinosinusitis in primary care management from 2013 claimed that there was little evidence to date to recommend the use of herbal remedies and compounds in treating ARS [Foden et al. 2013].

A Canadian survey has found that approximately 15% of patients with chronic rhinosinusitis use complementary and alternative medicines as treatment [Rotenberg and Bertens 2010].

The aim of this study was to present and analyse recent studies on herbs and substances used in the phytotherapy of rhinitis and rhinosinusitis as the main or complementary therapy.

In vitro research

As mentioned before, inflammation is one of the main mechanisms of acute and chronic sinusitis or rhinosinusitis. If it persists, the condition may lead to recurrences or even exacerbation of the disease [Hatipoğlu and Rubinstein 2008]. There are available drugs with an anti-inflammatory effect [Foden et al. 2013, Seidman et al. 2015], but there is always a risk of adverse effects. Some studies tried to evaluate the anti-inflammatory potential of substances present in medicinal herbs used as monotherapy or adjuvant therapy. Most available studies are based on *in vitro* models.

The first in this group of research was focused on determination of the anti-inflammatory properties of ethanolic extracts of *Inula helenium* L. and *Grindelia squarrosa* Pursh. It was confirmed *in vitro* that extracts of these plants may suppress neutrophil binding to the epithelium by regulation of β2 integrin and suppress IL(interleukine)-8. Importantly, these plants had an effect on TNF-α (tumour necrosis factor α) and IL-1β comparable with that of budesonide [Gierlikowska et al. 2020].

Xanthium strumarium L. methanolic extract was found to suppress production of nitric oxide and prostaglandin E2 and inhibit PDK1 kinase activity [Hossen et al. 2016a], while another research demonstrated that the extract may decrease the production of IL-1β, IL-6, and TNF-α. It influences the inflammatory signalling MAPK (mitogen-activated protein kinase) and AP (activator protein)-1 [Hossen et al. 2016b].

Ethyl acetate extract from *Angelica dahurica* Fisch., a herb commonly used in traditional Korean medicine, was found to inhibit LPS (lipopolysaccharide)-induced NO, PGE2 (prostaglandin e), and TNF-α production as well as expression of iNOS (inducible nitric oxide synthase) and COX-2 (cycloxygenase) by blocking phosphorylation of MAPKs, following I-κBα degradation and NF (nuclear factor)-κB activation [Kang et al. 2007].

Houttuynia cordata Thunb. ethanolic extract is considered useful in treatment of Th2-mediated or allergic inflammation by down-regulating the production of Th2 cytokines, TARC (Thymus- and activation- regulated chemokine)-induced migration, and basal migration of Jurkat T cells [Lee et al. 2008].

Monoterpene 1,8-cineole (eucalyptol) decreased the release of LPS-stimulated cytokines relevant for inflammation. In lymphocytes, it inhibited TNFα, IL-1β, IL-4, and IL-5 *in vitro*. Knowledge of this substance should be expanded to establish its potential use as adjunctive therapy in inflammatory airway diseases [Juergens et al. 2020].

Although it does not have significant anti-inflammatory properties, *Cedrus deodara* Roxb essential oil exhibits antifungal activity. Therefore, it can reduce inflammation in the respiratory system [Kumar et al. 2020].

Myristica fragrans Houtt. was studied to compare the properties of its ethanolic and water extracts. The latter was found to have better antioxidant, anti-inflammatory (inhibition of NO, TNF-α, and IL-6), and antimicrobial properties (especially against *Streptococcus pyogenes*, but also *Staphylococcus aureus* and *Candida albicans*) [Dechayont et al. 2020].

***In vivo* studies**

Astragalus membranaceus Schischkin water root extract was probably involved in reduction of the levels of inflammatory cells in nasal lavage fluid, number of eosinophils in the nasal mucosa, and IgE, IL-4, IL-5, and IL-13 levels in serum

by increasing the level of CD4+ CD25+ Foxp3+ T cells and through inhibition of NF-κB [Bing et al. 2019].

Water extract of roots of *Rumex patientia* L. was found to reduce inflammation in laboratory rats [Süleyman et al. 1999].

Ecballium elaterium L., which is applied in traditional medicine for rhinosinusitis, was tested in laboratory rats to check antibacterial properties of its extract. The extract of the plant exhibited anti- inflammatory activity and reduced fibrosis but also caused severe epithelium loss in the first hour after application. This indicates a need of further research to establish the safe and efficient dose and the duration of treatment [Eti et al. 2018].

Cyclamen europaeum L. water extract administered with an oral antibiotic during chronic rhinosinusitis exacerbation contributed to reduction of sinonasal symptoms and decreased the number of CRS recurrences [Lopatin et al. 2018].

In acute bacterial sinus infection, it was found that the herbal drug prepared from the roots of *Pelargonium sidoides* DC. had a better effect than a placebo [Bachert et al. 2009]. However, the drug should not be administered instead of antibiotics due to the risk of complications of bacterial infection.

Lyophillized extract of the *Alpine cyclamen* L. was analysed to establish its efficacy in treating acute rhinosinusitis. Saponins contained in this drug were found to reduce the surface tension on the nasal mucosal cells while simultaneously stimulating the trigeminal nerve receptors. This led to increased production of seromucous secretion and extensive drainage of the nasal and sinus cavities. Therefore, this plant may be useful in treating symptoms of acute rhinosinusitis [Jurkiewicz et al. 2016].

Given the number of positive signals on its effectiveness in allergic rhinitis, *Astragalus membranaceus* should be a subject of further larger multicentre trials [Matkovic et al. 2010].

Andrographolide and some of its synthetic analogues can inhibit the formation of bacterial biofilms, production of virulence factors, and adhesion between bacteria and cause destruction of bacterial integrity. It should be checked whether it is possible to achieve an enhanced effect by administration of the substance in combination with antibiotics, which may partially restore antibiotic susceptibility and increase antibiotic resistance [Ma et al. 2012, Guo et al. 2014, Tan et al. 2016].

Berberine was reported to reduce allergic reaction substantially in laboratory animals. Researcher assume its mechanism of action via CD4+CD25+Foxp3+ Treg cells by increasing their numbers and altering their function [Kim et al. 2015].

The efficacy of *Nigella sativa* extract against rhinosinusitis caused by *Staphylococcus aureus* was compared with that of the antibiotic cephalexin. The level of NO was reduced in both the cephalexin and *N. sativa* groups. This may mean the ability of this extract to prevent histopathological changes in rhinosinusitis [Yoruk et al. 2017].

Administration of *Rosae multiflorae fructus* hot water extract was reported to reduce eosinophil infiltration, mucus accumulation, goblet cell hyperplasia, collagen fibre deposits, and TNF-α, IL-4, and IL-6 levels [Song et al. 2016].

The aim of another study on the antimicrobial activity of plants was to determine a potential difference between the effects of Tsang-Erh-San extract granules with *Houttuynia* extract powder and erythromycin in treatment of CRS without nasal polyps. The research showed no significant difference in the outcomes of the treatment [Jiang et al. 2012].

Conclusion

To sum up, there is limited evidence that herbs and herbal medicines should be the main part of rhinitis and rhinosinusitis treatment. Especially when classic medicines like antibiotics are indicated, these herbal substances can only act as adjuvant treatment to relieve some of the disease symptoms. The researches analysed in this paper agree that the knowledge of some of the medicinal plants should be expanded and that more long-term follow-up studies should be performed to determine the effectiveness of herbal treatment. The results of tests carried out in experimental models give hope for the future that some known herbs can become the basis of new treatment with less adverse effects than standard medicines.

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Fitoterapia zapalenia błony śluzowej nosa i zatok przynosowych – przegląd aktualnego piśmiennictwa

Streszczenie: Zapalenie błony śluzowej nosa i zatok przynosowych to najczęstsze choroby górnych dróg oddechowych. Mimo najczęstszej etiologii wirusowej, najczęściej leczone są przy pomocy antybiotyków. Jasne wytyczne dotyczące terapii preparatami ziołowymi mogą poprawić efekt leczenia i zmniejszyć ryzyko działań niepożądanych. Celem tej pracy była prezentacja dostępnych badań dotyczących fitoterapii zapalenia błony śluzowej nosa i zatok przynosowych (alergicznego i infekcyjnego).

Nie przeprowadzono jak dotąd wiele badań na modelach ludzkich, ale dostępne prace sugerują możliwość naukowego potwierdzenia skuteczności terapii ziołowych. Istnieje potrzeba przeprowadzenia kolejnych badań dotyczących efektywnej dawki, wskazań i możliwych działań niepożądanych substancji uzyskanych z roślin.

Słowa kluczowe: alergiczny nieżyt nosa, zapalenie zatok przynosowych, zioła lecznicze

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Herbs in dentistry – overview of recent research

Summary. Herbal products and medicines have been commonly used for centuries all over the world, especially to treat basic health problems. Nowadays, there is a need to determine potential benefits of herbal therapy to human health and identify plants that provide biologically active substances for treatment of diseases that are normally treated with synthetic products. The aim of this study was to analyse available recent investigations of plant-derived substances used in treatment of oral cavity diseases. Some of the compounds have known anti-inflammatory or antibacterial properties, but not all have been clinically tested in human models. Clinical guidelines may improve patients' quality of life and the outcomes of treatment with elimination of most adverse effects of drugs.

Key words: dentistry, medicinal herbs, caries, mucositis, periodontitis

Introduction

Herbal and natural products have been used all over the world for centuries. Modern science has developed novel drugs with clinically proven efficacy but with a risk of potential adverse effects. Some substances obtained from plants have experimentally proven effects. As indicated by Kumar et al. [2013], the WHO reports that approximately 80% of people use traditional or herbal medicines to treat basic health problems and that ca. 25% of drugs in developed countries are composed of herbal ingredients or their derivatives. Clear guidelines on herbal therapy may improve the treatment outcomes. Herbal medicines are used in the prophylaxis and treatment of many stomatological diseases.

The aim of this study was to analyse recent studies of biologically active plant-derived substances and herbal therapies that can be used in oral cavity diseases.

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Dental caries

Dental caries is one of the most frequent diseases treated in dentist practices. It is mostly caused by *Streptococcus mutans* bacteria [Yadav et al. 2016, Ravi et al. 2017, Karadağlıoğlu et al. 2019]. It can disrupt the chewing process and cause aesthetic defects [Karadağlıoğlu et al. 2019].

Microorganisms like *Lactobacilli* spp. and *Candida albicans* are involved in its progression [Karadağlıoğlu et al. 2019]. It progresses over time as a result of the interaction between the host, diet, and oral cavity microflora. The essence of the disease is the destruction of enamel, dentin, and cementum. The disease affects patients all over the world [Thomas et al. 2017]. Since dental caries cavities are irreversible, research should concentrate on prevention of the disease. Early stages of caries (incipiens) can be reversed or prevented from progression. Fluorine compounds are the key elements in the prophylaxis with proven efficacy. Unfortunately, synthetic chemicals can cause serious side effects. Hence, there is a need to continue investigations of less toxic alternatives [Karadağlıoğlu et al. 2019].

As demonstrated in an *in vitro* study, mango and eucalyptus extracts exhibited antibacterial properties (lower than chlorhexidine and ciprofloxacin), while extracts of mint leaves and garlic inhibited microbial growth only at higher concentrations [Ravi et al. 2017]. The activity of *Origanum dubium* L. and *Cinnamomum cassia* L. oils against *Streptococcus mutans* was determined by comparison of the efficacy of herbal toothpastes with and without addition of these oils. Carvacrol, thymol, p-cymene, and γ-terpinene contained in *O. dubium* exhibited antibacterial, antifungal, and antioxidant activity, while *C. cassia* exerted an antibacterial effect thanks to its content of cinnamaldehyde [Karadağlıoğlu et al. 2019].

Achyranthes aspera L. root and stem extracts showed antibacterial activity in an *in vitro* study, probably associated with the content of alkaloids and tannins. Its lowest concentration showing inhibition of the growth of *Staphylococcus aureus*, *Streptococcus heamolyticus*, *Escherichia coli*, and *Bacillus typhosus* was 2.5%. At a higher concentration, it had a similar *in vitro* efficacy to chlorhexidine (chlorhexidine inhibited the growth of the tested microorganisms at a concentration of 0.2%). These plant extracts should be further tested, since they exert less adverse effects [Yadav et al 2016].

In a randomized double-blind controlled clinical trial, mouth rinse composed of green tea, lime, and garlic was reported in a study from 2017 to be nearly as effective against *Streptococcus mutans*, *Lactobacillus* spp., and *Candida albicans* as a sodium fluoride rinse. Green tea is very rich in fluoride and bacteriostatic catechin and can have an anticariogenic effect by mediating the activity of protective salivary components. Garlic can act as an antiviral, antifungal, and bacteriostatic agent against *Streptococcus mutans* thanks to the content of allicin and thiosulphonates [Thomas et al. 2017].

Herbal treatment in endodontics

The complex anatomy of root canals causes difficulties in maximum disinfection, which is the main goal of root canal treatment. Bacteria, especially anaerobic species, with the most frequent presence of *Enterococcus faecalis*, are the main agents of pulpo-periapical diseases, sometimes with coexistence of *Candida albicans* [Noushad et al. 2018]. It is possible to achieve bacterial reduction mainly through irrigation, intra-canal medicaments, and partly through mechanical shaping of the canals. Sodium hypochlorite (NaOCl), ethylene-di-amine-tetra-acetic acid (EDTA) solutions, and chlorhexidine (CHX) are used most frequently to reduce bacterial counts, eliminate bacterial biofilms, remove the intracanal smear layer, and neutralise toxins produced by infection agents. Despite their indisputable effectiveness, they have possible significant adverse effects. Therefore, it is important to develop substances with similar anti-microbial activity but less undesirable properties. The efficacy of herbal antimicrobial substances in root canal treatment in comparison with medicines used commonly in dentistry was tested. It was found in an *in vitro* study that, despite its anti-microbial properties, *Aloe vera* L. is not recommended for use in root canal irrigation [Sahebi et al. 2013]. Its extract, along with cashew, apple, papaya leaf, and guava leaf extracts had significantly lower antimicrobial activity tested *in vitro* [Noushad et al. 2018]. Probably thanks to the presence of flavonoids, propolis has shown some antibacterial activity but no antifungal effect. Its indications in dentistry should be further investigated [Tyagi et al. 2013, Bhandari et al. 2014].

Post extraction socket healing

Although it is a common procedure in dentistry, tooth extraction poses a significant risk of complications, e.g. pain, swelling, pus drainage, and dry socket. The standard procedure includes postoperative antibiotic therapy, which may cause some adverse effects [Melo Júnior et al. 2002]. Dry socket appears when the clot is either not formed or dislodged accidentally by rinsing [Nimma et al. 2017].

Due to the increasing popularity of *Aloe vera* L. in medicine and dentistry, its effectiveness in the healing of post extraction sockets was tested. A cross-sectional randomized interventional study conducted on 40 patients demonstrated that the extract from this plant improved postoperative wound healing and decreased the severity of symptoms, compared to only analgesic treatment [Lone et al. 2018].

Schinus terebinthifolius extract was tested *in vitro* and *in vivo* in rat models in comparison with gentamicin. It was found that it had a similar antibacterial effect to that of the antibiotic but less adverse effects. This issue, however, needs more research. The extract was also found to improve clot formation and wound healing [Nimma et al. 2017].

Curcumin also is known to improve wound healing and possess antimicrobial properties. A randomised clinical study from 2018 revealed that curcumin improved the outcome of dry socket treatment and reduced discomfort, pain, and the inflammatory process.

Periodontitis

Periodontitis is an inflammatory disease that leads to destruction of connective tissue supporting teeth, i.e. the gum, periodontal ligament, cementum, and alveolar bone. The main cause of this disease is the growth of anaerobic dental plaque-forming bacteria and prolonged exposure of connective tissue to inflammation, neutrophil infiltration, and enzymes like PGE2 as well as IL-6, IL-8, and TNF-α. It can even lead to tooth loss [Kharaeva et al. 2020].

An important part of periodontitis treatment is the use of antibacterial substances. Chlorhexidine, fluorides, xylitol, triclosan, and their combinations have a proven antibacterial, anti-caries, and anti-inflammatory effect [Kharaeva et al. 2020], but they can cause adverse effects like most synthetic products. Some studies suggest that herbal products can have similar efficacy with significantly fewer side effects [Geidel et al. 2017]. As demonstrated in the studies by Asahi et al. [2014] and Araghizadeh et al. [2013], green tea can inhibit the growth of *Porphyromonas gingivalis* and *Prevotella spp.* bacteria (*in vitro* studies). Additionally, extracts from green tea reduced bone resorption in animal models by suppressing RANKL (Receptor Activator for Nuclear Factor κ B Ligand), cyclooxygenase-1, and cytokine PGE2. This substance needs further *in vivo* research in humans.

Triphala (powder composed of *Emblica officinalis* L., *Terminalia chebula* Retz., and *Terminalia belerica* Gaertn.) was proved in clinical trials to have antiplaque and anti-inflammatory activity similar to that of chlorhexidine. It can also inhibit *Streptococcus mutans* growth and reduce the activity of collagenase responsible for destruction of connective tissue [Naiktari et al. 2014, Abraham et al. 2005].

Rubia cordifolia L. possesses anti-inflammatory properties. Mollugin, i.e. the main component of this plant, is able to inhibit RANKL-induced osteoclast differentiation and decrease the activity of osteoclasts [Zhu et al. 2013].

Piperine contained in *Piper nigrum* L. and *Piper longum* L. plants was found to delay alveolar bone loss in an animal model. It can act as a free radical scavenger and reduces the level of TNF-α [Dong et al. 2015].

The best results were reported in a comparative clinical study conducted by Kharaeva et al. [2020], who analysed combinations of herbal compositions with classic synthetic treatment. They found that addition of herbs could enhance regular treatment and allowed reduction of the doses of potentially toxic substances.

Oral mucositis

Oral mucositis involves inflammatory destruction of the lining of the oral cavity, impairing patient's quality of life. It occurs most often after chemo- or radiotherapy [Kono et al. 2014] but also due to infection, the action of physical, chemical, and thermal factors, immunological or systemic diseases, trauma, neoplasia, and chronic use of tobacco and alcohol. Delay or discontinuation of the systemic treatment may be necessary due to mucosal infection and compromised immunity. The main symptoms are erythema and/or ulceration of oral mucosa caused by oxidation and apoptosis induced by nitric oxide (NO), cyclooxygenase (COX), protein kinases, cytokines, and nuclear factors as well as genetic-based risk factors like epigenetic changes in DNA methylation [Kono et al. 2014].

The frequent treatment methods and medicines include debridement, disinfection, topical or systemic analgesics, prevention, and control of bleeding. Many agents, e.g. alopurinol, chlorhexidine, diphenhydramine, aluminium hydroxide, and pallifermin, have been used to prevent or lessen the symptoms and signs of oral mucositis [Kono et al. 2014]. However, due to their adverse effects, development of less toxic herbal products would improve the general outcome of the disease and patients's quality of life.

In the analysed papers, *Aloe vera* extract was found to reduce progression of oral mucositis and improve patients' quality of life, probably thanks to its antioxidant properties, COX-2 suppression, and immunomodulatory effect [Puataweepong et al. 2010]. A mouthwash containing *Camellia sinensis* L. leaf extract acted probably via neutralisation of the excessive production of Reactive Oxygen Species (ROS) and inhibition of ulcerative destruction in oral mucositis [Carulli et al. 2013]. *Acacia catechu* L. extract exerted a promising healing effect, which should further be tested [Shi and Shan 2009].

In a randomized, double-blind clinical trial, a chamomile mouthwash was found to have the same efficacy as that of an allopurinol mouthwash in the prevention of chemotherapy-induced stomatitis [Shabanloei et al. 2009]. Moreover, a mouthwash containing 1% *Chamomilla recutita* L. extract was effective against mucositis in adult patients receiving allogeneic haematopoietic stem cell transplantation [Braga et al. 2018].

Two studies confirmed the efficacy in prevention of chemo- and radiotherapy-induced oral mucositis of the Hangeshashinto herbal mixture (Extracts of *Pinellia Tuber* Thunb., *Scutellarai Radix* L., *Glycyrrhizae Radix* L., *Ziziphi* P. *Miller Fructus*, *Ginseng C.A. Meyer Radix*, *Zingiberis Processum* Mill. *Rhizoma*, *Coptidis Franch. Rhizoma*). It acted by direct inhibition of PGE-2 production, COX-2 inhibition, and prevention of neutrophils from infiltrating the mucosa [Kono et al. 2014, Yamashita et al. 2015].

Lonicerae flos and *Glycyrrhizae radix* both diminished symptoms of mucositis due to their anti-inflammatory and antioxidant effect (suppression of

interleukin 1-β (IL-1β), IL-6, LPS-induced NF-κB, and COX-2 gene expression) [Bao et al. 2008].

Peppermint ethanolic extract was found to have anti-fungal and antibacterial properties and alleviate symptoms effectively [Ashktorab et al. 2010].

Conclusion

Summing up, some plants and herbal extracts showed therapeutic activity in oral cavity diseases. Most of the tested substances exerted an anti-inflammatory effect, and some may be used in the future as adjunctive therapy to prevent pathogenic bacteria from growing and forming dental plaque. Some of the extracts have immunomodulatory, antitoxic, antiseptic, or sedative effects. Especially, the enhancement of wound healing can be a useful effect. Such plants as *Aloe vera* or green tea show promising effects in oral mucositis or post-extractive wound healing, but their efficacy needs further double-blind randomized clinical trials in humans.

Currently, they should not be used in monotherapy or instead of substances with a proven therapeutic effect. Considering the possible adverse effects of synthetic medications, researchers should concentrate on finding the range of diseases where switching from conventional treatment to herbal medicines would do no harm to the patient but exert a similar therapeutic effect and fewer adverse effects.

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Zastosowanie ziół w stomatologii – przegląd aktualnego piśmiennictwa

Streszczenie. Preparaty i leki ziołowe są używane od wieków na całym świecie, głównie w leczeniu podstawowych problemów zdrowotnych. Istnieje potrzeba doświadczalnego określenia potencjalnych skutków zdrowotnych terapii ziołowych dla ludzkiego zdrowia oraz sprawdzenia, które substancje aktywne zawarte w roślinach mogą zostać użyte do terapii chorób leczonych aktualnie przy użyciu leków syntetycznych. Celem tej pracy była analiza publikacji na temat zastosowania substancji zawartych w roślinach w leczeniu chorób jamy ustnej. Niektóre ze znanych związków posiadają udowodnione działanie przeciwzapalne i przeciwbakteryjne, jednak nie wszystkie zostały poddane badaniom klinicznym u człowieka. Wytyczne dotyczące leków ziołowych mogą poprawić jakość życia pacjentów i efekty leczenia przy jednoczesnym minimalizowaniu ryzyka działań niepożądanych.

Słowa kluczowe: stomatologia, leki ziołowe, próchnica, mucositis, periodontitis

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Importance of bioactive substances of plant origin for the health and production performance of dairy cattle

Summary. Plant bioactive substances are successfully used for the prophylaxis and therapy of many diseases and for enhancement of the production performance in farm animals. Based on original scientific papers, the importance of some phytobiotics in rearing dairy cattle is discussed here. Plant phytobiotics administered as single herbs or herbal mixtures are a valuable dietary component with multidirectional effects on all animal systems. They have been widely applied for regulation of digestive processes, stimulation of immunity and health in calves, and support of lactation and udder health in cows.

Key words: phytobiotics, therapy, prophylaxis, dairy cattle, calves

Introduction

Medicinal plants used for supplementation of farm animal diets can be an alternative in the treatment and prevention of diseases and can support immunity, but their desirable effect is attainable only upon a long-term regular application [Walkenhorst et al. 2020]. The use of phytobiotics in ruminants reduces the growth of harmful microorganisms, improves rumen fermentation efficiency, and reduces methane emission [Oskoueian et al. 2013, Petrič et al. 2020]. Additionally, herbs contain many valuable ingredients improving the quality of animal products [Park et al. 2018].

The aim of the study was to present the importance of some phytobiotics in dairy cattle farming based on original scientific publications.

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Phytobiotics in the regulation of digestive processes in dairy cattle

The effects of the use of herbs containing phytobiotic substances, e.g. linalool (*Coriandrum sativum*), cineole (*Amomum subulatum*, *Rosmarinus officinalis*), trigonelline (*Trigonella foenum-graecum*), anethole (*Pimpinella anisum*), and thymol (*Thymus vulgaris*), are reflected in the regulation of digestive processes in animals, enhancement of enzyme and bile secretion, and stimulation of appetite. They also promote detoxification of the organism and improve absorption of nutrients from feed [Santos et al. 2015, Froehlich et al. 2017]. Essential oils, pigments (carotenoids and anthocyanins), alkaloids, glycosides, phenolic acids, phytosterols, and flavonoids contained in medicinal plants stimulate the immune system, improve blood circulation, increase appetite, stimulate digestion, and exert anti-inflammatory [Kekana et al. 2020], antimicrobial [Di Pasqua et al. 2006], and antioxidant [Fasseas et al. 2008] effects.

Garlic oil and its component alliin have been shown to limit methane production by inhibition of the growth of methanogens [Hart et al. 2006]. It has also been demonstrated that *Cosmos bipinnatus* Cav., which is a source of phenols and their derivatives (tannins), exerts antimethanogenic effects *in vivo* [Hernández-Pineda et al. 2018] in dairy cattle. As reported by Oskoueian et al. [2013], flavonoids such as quercetin and naringin reduce the population size of protozoa and methanogens. They inhibit the synthesis of microbial cell walls, cytoplasmic membrane, and nucleic acids, which contributes to a decrease in CH_4 production.

Petrič et al. [2020] have demonstrated that a mixture of wormwood, chamomile, common fumitory, and mallow has potent antioxidant activity in the rumen and potential to reduce CH_4, and ammonia emissions and concentrations. The efficiency of fermentation in the rumen and nutrient utilization can be improved by oregano (*Origanum vulgare* L.) [Akbarian-Tefaghi et al. 2018], lemongrass (*Cymbopogon citratus* (DC.) Stapf.), and peppermint (*Mentha* × *piperita* L.) [Wanapat et al. 2013].

Potential use of phytobiotics for treatment and prophylaxis in calves

The antibacterial, anti-diarrhoeal, anti-inflammatory, and immunomodulatory effects of garlic can be useful in the prophylaxis and treatment of acute calf diarrhoea. Allicin contained in the plant has been shown to exert anti-diarrhoeal effects by reduction of the number of faecal coliform bacteria [Ghosh et al. 2011]. The immunomodulatory activity of *Allium sativum* L. may also support immune defence in immunocompromised calves [Kekana et al. 2020].

Polysaccharides, alkylamides, caffeic acid esters, and polyacetylenes are the main compounds contained in *Echinacea* Moench [Barnes et al. 2005]. An *in vivo* study [Seckin et al. 2018] has demonstrated improved immune response in calves

receiving a medicinal preparation based on *Echinacea purpurea* (L.) Moench and *Pelargonium sidoides* DC.

The antitussive and mucolytic effects of thyme are associated with the content of essential oil composed of thymol and its isomer carvacrol [Kachur and Suntres 2020]. Animal studies have shown improvement of mucociliary clearance [Wienkötter et al. 2007], anti-inflammatory properties [Vigo et al. 2004], and antibacterial activity [Stojković et al. 2013] in the respiratory system. As shown by Kissels et al. [2017], both thymol and carvacrol have an additive effect in combination with tilmicosin against microorganisms isolated from the respiratory tract of calves. A similar effect was observed after administration of a combination of thymol with doxycycline.

Chinese tea contains large amounts of such phytobiotics as polyphenolic compounds (up to 25% of catechin derivatives in unfermented plants), purine alkaloids (caffeine, theobromine, theophylline), and flavonoids. Hence, it can be effective in the treatment of diarrhoea, stimulation of the immune system [Hiller and Melzig 2010], and control of the flu virus [Smee et al. 2011]. It has been proven [Katsoulos et al. 2017] that preventive effect against neonatal diarrhoea syndrome was also exerted by daily administration of oregano essential oil in calves for the first 10 days of their life.

The root of liquorice is used for therapeutic purposes due to the content of its main active phytobiotic – glycyrrhizin saponin [Hiller and Melzig 2010]. *In vivo* studies have demonstrated that liquorice has immunostimulatory effects (stimulation of cellular and non-specific response) [Borsuk et al. 2011], antitussive activity [Saha et al. 2011], relaxative effects on tracheal smooth muscles [Liu et al. 2008], and a regulatory effect on the gastrointestinal tract [Chen et al. 2009]. Other studies demonstrate its antimicrobial [Kim et al. 2002] and antiviral activity via activation of autophagy [Laconi et al. 2014]. Hence, liquorice may be beneficial in the prophylaxis and treatment of respiratory and gastrointestinal diseases caused by viruses, including coronaviruses [Cinatl et al. 2003].

Galactopoietic phytobiotics

The best-known lactation-supporting plant is the milk thistle (*Silybum marianum* L.) containing e.g. silymarin, the use of which in dairy cows contributed to an increase in the daily milk yield by 3–4 kg in various studies [Garavaglia et al. 2015, Ulger et al. 2017] and a reduction of the number of somatic cells in milk [Garavaglia et al. 2015]. Fenugreek (*Trigonella graecum foenum* L.) is characterized by galactopoietic activity as well. It was found [Abo El-Nor et al. 2007] that the daily consumption of 200 g of fenugreek seeds resulted in a 1–1.5 kg increase in the daily milk yield in buffalo females, with a simultaneous increase in the protein and lactose content. In dairy cows, the addition of 20% d.w. of seeds to the feed dose did not significantly increase milk yield but contributed

to an increase in fat content, a decrease in cholesterol level, and a change in the fatty acid profile [Shah and Mir 2004]. Due to its ability to inhibit *Pseudomonas* and *Escherichia coli* growth, fenugreek can be an element of *mastitis* treatment [Jatav et al. 2019]. A wild species *Asparagus racemosus* Willd. containing steroid saponin phytobiotics can also be used for enhancement of lactation in cows. As shown by Saini et al. [2018], addition of powdered roots of this plant at a dose of 40–120 mg/animal/day increased the milk yield (by 0.73–2.63 kg) and the content of fat and proteins in milk.

Phytobiotic-aided prevention and therapy of *mastitis*

With their bactericidal and bacteriostatic properties, anti-inflammatory and antifungal activity, phagocytosis-stimulation effects on bacteriophages, and support of the immune system, plant bioactive substances can be used in prevention and treatment of udder inflammation (*mastitis*) in cows. The widest spectrum of *in vitro* activity against *mastitis* pathogens was demonstrated by alkaloid-, tannin-, and steroid-rich ethanol extracts from the aboveground parts of *Lepidium virginicum* L. [Macías Alonso et al. 2020]. A positive effect in *Staphylococcus aureus*, *Shigella* spp., and *Salmonella* spp. infections was exerted by *Brickellia veronicaefolia* Kunth extracts containing flavonoids, tannins, and terpenoids. Antibacterial activity of alkaloids, flavonoids, and saponins contained in crude extracts from *Allium sativum* L., *Bunium persicum* (Boiss.) B. Fedtsch., *Oryza sativa* L., and *Triticum aestivum* L. against *S. aureus*, *E. coli*, and *Klebsiella pneumoniae* was reported by Amber et al. [2018]. High content of polyphenols with strong antibacterial activity against pathogens isolated from milk of *mastitis*-affected cows was detected in alcoholic extracts of *Artemisia absinthium* L. [Pașca et al. 2017]. Stagos et al. [2012] reported that water extracts from *Mentha pulegium* L., *Sideritis raeseri* Boiss. et Heldr. *ssp. raeseri* and three *Salvia* species: *S. pomifera* L. ssp. *calycina* (Sm.) Hayek., *S. pomifera* L. ssp. *pomifera*, and *S. officinalis* L. exerted a strong inhibitory effect on *S. aureus.* In turn, Hu et al. [2001] showed that saponin extracts from ginseng roots (*Panax ginseng* CA Meyer), produced commercially by Indena® (Indena SPA, Milan, Italy), with saponin content equivalent to 14% ginsenoside Rg_1, had a beneficial impact on udder health when administered via subcutaneous injections.

Conclusion

Based on the current knowledge and results from animal experiments, it can be concluded that medicinal plants may be an excellent alternative to antibiotics and synthetic chemicals used in dairy cattle breeding for achievement of better production results. Due to the abandonment of grazing animals in many farms,

single herbs or their mixtures are used in nutrition as fresh or dried additives, extracts, infusions, decoctions, macerates, and essential oils. Herbal supplementation may bring positive effects, e.g. improvement of appetite and digestion by stimulation of the immune system, antimicrobial, antioxidant and anti-inflammatory effects, enhancement of productivity, and improvement of the overall health status. The inclusion of phytobiotic plants to diet should be considered thoroughly and take into account the properties of the active substances contained therein, the physiological needs of animals, and consumer expectations of the quality of animal products.

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Znaczenie wybranych roślinnych substancji bioaktywnych w aspekcie zdrowia i produkcyjności bydła mlecznego

Streszczenie. Roślinne substancje bioaktywne są z powodzeniem stosowane w profilaktyce i terapii wielu chorób oraz wspomaganiu produkcyjności zwierząt hodowlanych. Na podstawie wybranych oryginalnych prac naukowych omówiono znaczenie niektórych fitobiotyków w chowie bydła mlecznego. Roślinne fitobiotyki stosowane jako pojedyncze zioła lub ich mieszanki stanowią cenny składnik diety o wielokierunkowym działaniu na wszystkie układy zwierzęce. Znalazły szerokie zastosowanie w regulowaniu procesów trawiennych, stymulacji odporności i zdrowotności cieląt, wspomaganiu laktacji u krów i stanu zdrowotnego ich wymion.

Słowa kluczowe: fitobiotyki, terapia, profilaktyka, bydło mleczne, cielęta

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Biologically active compounds from herbs used in meat and meat products

Summary. Oxidative processes taking place during storage and cooking are one of the main causes of deterioration of the quality of meat and its products and pose a serious problem to food technologists. Polyphenols contained in herbs inhibit the development of undesirable microflora and limit oxidative changes in fats and proteins and determine the flavour and colour of meat and meat products.

Key words: thyme, rosemary, sage, marjoram, oregano, basil, phenolic antioxidants

Introduction

Herbaceous plants were used already in ancient times. Their flavour and therapeutic properties have been discovered and used over the centuries, making them an integral part of the modern human diet [Starek and Pawłat 2018]. Due to their content of polyphenolic compounds, herbs have become a common ingredient in many cooking recipes [Aminzare et al. 2019]. Polyphenols inhibit the development of undesirable microflora and limit oxidative changes in fats and proteins contained in muscle tissue and meat products, thereby extending the shelf life of raw meat material during storage [Sadowska et al. 2014, Hashemi et al. 2016]. Additionally, herbs exert a positive effect on the physiological and biochemical processes in the human organism, stimulate appetite, improve metabolism, digestibility of nutrients, and gastrointestinal function, prevent cancer, and strengthen immunity [Dudek-Makuch et al. 2019]. With their documented health-enhancing properties, herbal plants are regarded as functional food. Consumption of this type of food contributes to improvement of health and welfare and reduction of the risk of many diseases.

This literature review presents bioactive substances contained in some herbal plant species and recommendations for their use as additives to meat and meat products.

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Prevention of adverse changes in meat and meat products

Concern for consumer safety is associated with the necessity to extend the shelf life and ensure appropriate microbiological quality of food [Turyk et al. 2013]. Due to its chemical composition and water retention properties, meat represents food products with a limited shelf life. The main undesirable transformation occurring in meat and its products during storage is lipid oxidation. Additionally, frying and smoking promotes generation of free radicals in food. Oxidative deterioration of meat and its products results in the development of off-flavour, discoloration, formation of toxic compounds, loss of nutrients, and drip losses, which all result in a shorter shelf life. To limit these changes, synthetic antioxidants that scavenge or reduce free radicals and prevent reactions with oxygen are applied. Nowadays, their use has been limited due to the possible health risks and toxic effects of these synthetic compounds on human health as well as the increasing tendency towards consumption of natural components [Aminazare et al. 2019]. It is therefore reasonable that herbs containing phenolic antioxidants should be used.

Herbal plants as a source of bioactive compounds and mechanism of action of natural antioxidants

Several studies showed that extreme antioxidant activity has been found in herbs, spices, and extracts so they can be used as natural antioxidants in meat and meat products [Bozin et al. 2006, Agbor et al. 2007, Fasseas et al. 2008, Kong et al. 2010, Rather et al. 2018]. The majority of natural antioxidants are phenolic compounds (and the most important are flavonoids, phenolic acids), the compounds of essential oils, tocopherols and carotenoids [Kumar et al. 2015, Aminzare et al. 2019]. Carnosol, carnosic acid, and carvacrol derived from rosemary, thyme, and marjoram extracts are considered the strongest antioxidants [Gramza-Michałowska et al. 2008].

Some phenolics prevent the formation of free radicals and propagation of ROS, while other scavenge free radicals and chelate transition metals [Kumar et al. 2015, Rather et al. 2018]. Phenolic acids trap free radicals; flavonoids scavenge free radicals and chelate metals (Fe^{2+}, Fe^{3+}, and Cu^{2+}) as well [Bozin et al. 2006]. The antioxidant potential of these natural substances depends on their pattern of functional groups on this skeleton. For example, the number and position of free hydroxyl (–OH) groups on flavonoid skeleton decide the free radical-scavenging potential. Presence of multiple –OH groups and ortho-3, 4-dihydroxy structures enhance the antioxidant potential of plant-based phenolics [Rather et al. 2018]. Polymeric structures (containing more –OH groups) possess more antioxidant potential, whereas glycosylation of functional groups (reduction of –OH groups) usually decreases antioxidant effectiveness. Plant-derived pigments

(anthocyanins and their hydrolyzed products, anthocyanidins) also contain –OH groups, which can donate $H^{\cdot}$ and thus possess antioxidant properties. Some phenolics also contain vicinal –OH groups attached to aromatic ring. These phenolics donate $H^{\cdot}$ as well as vicinal –OH groups that can chelate metals, thus prevent oxidation via more than one method [Kumar et al. 2015, Rather et al. 2018]. Bioactive compounds present in herbs have the ability to inhibit many enzymes and microbial growth and can improve the taste of food [Zhang et al. 2010, Świąder et al. 2017, Wójcik-Stopczyńska and Jakubowska 2018].

Application of herbal biocompounds in meat processing

In the meat industry, fresh or dried and ground herbal leaves are primarily used. They can also be replaced by herbal extracts and essential oils [Hashemi et al. 2016]. The concentration of biologically active substances contained therein is approximately 30-fold higher than in fresh and dried spices [Uchman 2008]. Thyme, rosemary, sage, marjoram, oregano, basil, and savoury are the most popular herbal spices added to meat and its products. Their role in meat processing can be considered in several aspects. For instance, they have antioxidant and bactericidal properties and improve organoleptic properties, giving meat products a specific flavour, aroma, and desirable appearance [Naveena et al. 2013, Świąder et al. 2017]. Appropriate use of herbs is part of real culinary art indispensable for production of meat products with the desired flavour and aroma composition. Therefore, it is necessary to know the properties of herbs.

Thyme, also referred to as common thyme (*Thymus vulgaris* L.), has a specific pleasant but strong thymol aroma. It contains approximately 10% of tannins and essential oil (3.5–5.4% d.w.), with dominance of thymol (up to 80%) and carvacrol (up to 20%) in its chemical composition. Both thymol and carvacrol exhibit high antioxidant activity [Wójcik-Stopczyńska and Jakubowska 2018]. Other compounds with antioxidant activity include rosmarinic, ferulic, and gallic acids, phenolic diterpenes, luteolin, borneol, bornyl acetate, cineol, cymene, α-pinene, linalool, and linalool acetate [Dudek-Makuch et al. 2019]. Additionally, thyme herb contains saponins and flavonoids. It was shown that thyme reduced DPPH (2-diphenyl-1-picryl hydrazyl radical) radical formation, lipid peroxidation and the deterioration of sarcoplasmic proteins, helping to preserve the meat even after 2 wk of storage. Thyme essential oil exhibited very strong freeradical scavenging ability and inhibited Fe^{2+}/ascorbate and Fe^{2+}/ H_2O_2 induced lipid oxidation [Bozin et al. 2006]. Thyme has been used for centuries as a seasoning to extend the shelf life of meat. Its positive effect contributes to an increase in the antioxidant potential of meat dishes; in turn, thymol and carvol give meat products a characteristic strong aroma and a slightly spicy and refreshing flavour [Starek and Pawłat 2018]. Thyme suits venison and stewed meat best but can also be used to season liver, minced chops, roast meats, poultry, and fish.

Young rosemary leaves (*Rosmarinus officinalis* L.) contain 1.5% of essential oil. Its content is 1–2.5% in dried leaves and from 0.4 to 2% in the herb. The main components of rosemary essential oil include monoterpene hydrocarbons: camphor (14.5%), 1.8-cineol (12%), borneol (10.5%), α-pinene (8.5%), and camphene (7%) [Hać-Szymańczyk et al. 2009]. The oil has a strong herbal-wood aroma reminiscent of camphor and a sharp, bitter, spicy, and cooling flavour [Nowak et al. 2013]. The raw material contains the following antioxidant compounds: flavones, steroid diterpenes, triterpenes, rosmanol, epirosmanol, 9-ethylrosmanol, rosmariquinone, carnosol, genkwanin, galdosol, methyl carnosate, rosmarinic acid, caffeic acid, carnosic acid, ferulic acid, luteolin, tannins, bitter compounds, saponins, and resins [Dudek-Makuch et al. 2019]. These phytochemicals play an important role in inhibition of lipid peroxidation and substantially reduce the concentration of various amino glycosides. They also have antimicrobial, antiviral, antifungal, and preservative activity [Kowalska and Olejnik 2010]. Rosmarinic acid has been shown to have bactericidal and bacteriostatic activity against *Staphylococcus* sp., *Listeria* sp., and *Helicobacter pylorii* bacteria and *A. flavus*, *F. oxysporum*, and *P. cyclopium* fungi [Wójcik-Stopczyńska and Jakubowska 2018]. The addition of rosemary extracts to meat, frankfurters, sausages, and pies was found to slow down oxidation and rancidification significantly, thus preventing adverse oxidative changes, even in muscle tissue that had been freeze-stored for six months [Naveena et al. 2013, Świąder et al. 2017]. The antioxidant properties of rosemary are mainly associated with the presence of the strongest active ingredients, i.e. rosmarinic and carnosic acids, carnosol, epirosmanol, and methyl carnosate [Naveena et al. 2013, Starek and Pawłat 2018]. Rosemary extract is the most effective antioxidant preserving the colour of meat. It also exerts the most beneficial protective effect on thiamine contained in dried meat [Dudek-Makuch et al. 2019]. Rosemary has been included in the list of additives as an antioxidant (E 392). Rutin contained in rosemary chelates metal ions [Wereńska 2013]. Rosemary oil has been reported to have strong antibacterial activity against *Enterococcus faecalis*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus subtilis*, and *Klebsiella pneumoniae* [Kowalska and Olejnik 2010]. It is used as a fragrant substance in the production of canned food and meat and fish dishes. Verbenone and sesquiterpene compounds are carriers of the characteristic rosemary aroma [Kozłowska-Lewecka and Borowiecka 2011].

The content of essential oil in sage (*Salvia officinalis* L.) leaves is in the range of 2.5–3%, and α- and β-thujone are its main component. Sage essential oil contains numerous terpenes, e.g. camphor, cineol, borneol, and pinene. Its activity is higher than that of synthetic antioxidants [Mata et al. 2007]. Other important antioxidant compounds present in sage include rosmarinic acid, carnosol, carnosolic acid, carnosic acid, rosmanol, rosmanal, epirosmanol, methyl carnosate, luteolin, tannins, bitter compounds, resins, vitamin A saponins, and

malic, fumaric, and nicotinic acids [Woźniak et al. 2009, Dudek-Makuch et al. 2019]. Carnosol and carnosic acid are the most potent active ingredients in sage extracts. These compounds are responsible for 90% of the antioxidant properties of herbal preparations. They play an important role in inhibition of lipid peroxidation and significantly reduce the concentration of various aminoglycosides. Sage extract has antioxidant activity in fresh and cooked meat and in cured meat homogenates [Dudek-Makuch et al. 2019]. During refrigerated storage, sage-containing sausages showed significantly retarded increases in TBARS (Thiobarbituric Acid Reactive Substances) values and in the formation of protein carbonyls. Sage was also found to retard the deterioration of colour and texture during storage [Zahng et al. 2013]. *S. officinalis* is most often recommended as an addition to cooked, grilled, or roasted meats (poultry, pork, veal, lamb, mutton, and venison). It is ideal for delicate stuffing, paté, liver, and sausages. The use of sage as a seasoning for fatty meats and dishes has health benefits, in addition to its sensory value, as the herb stimulates and facilitates digestion [Woźniak et al. 2009].

Marjoram (*Origanum majorana* L.), like other spices, can be added to food as fresh, dried, crushed herb or its extracts. It contains 2–3% of essential oil composed of terpinol, citral, linalool, eugenol, terpinolene, and flavonoids [Seidler-Łożykowska et al. 2006]. The raw material (flowers and leaves) contains bitter compounds, tannins, vitamins, and mineral salts. Antioxidant compounds present in marjoram include carvacrol, rosmarinic acid, eugenol, phenol, ascorbic acid, ursolic acid, and oeanolic acid. Marjoram has been reported to have high DPPH scavenging capability [Newerli-Guz 2012]. Gamma-terpinene contained in the *O. majorana* oil has antibiotic properties against certain bacterial genera and is effective against *C. albicans* yeast. Marjoram added to stewed, fried, roasted, and grilled meat, fatty foods, sandwich meats, sausages, meat paté, lard, poultry, and fish is responsible for their strong, extremely aromatic smell and bitter spicy flavour. The seasoning protects these products from oxidative transformations due to its content of essential oils and polyphenols (mainly quercetin, rutin, and quercitrin). Sabinene cis-hydrate is the carrier of the characteristic aroma of marjoram [Kozłowska-Lewecka and Borowiecka 2011]. Marjoram can replace salt in a salt-free diet [Newerli-Guz 2012].

Oregano herb (*Origanum vulgare* L.) contains up to 3% of essential oil, which is rich in such phenols as carvacrol and thymol, in addition to thymol acetate and p-cymene [Wójcik-Stopczyńska and Jakubowska 2018]. The raw material contains numerous antioxidant compounds: phenolic acids (p-hydroxybenzoic, vanillic, caffeic, o- and p-coumaric, ferulic, gallic, rosmarinic, chlorogenic, and dihydroxy-caffeic acids), flavonoids (apigenin, luteolin, quercetin, kaempferol, eriodictyol, naringenin), sesquiterpenes, and catechin [Wereńska 2013, Dudek-Makuch et al. 2019]. Essential oils and extracts are active against a wide spectrum of bacteria and moulds, e.g. *E. coli*, *Enterobacter*, *Bacillus*, *Listeria monocytogenes*,

S. aureus, *Candida*, *Fusarium*, *Aspergillus*, *Rhizopus*, and *Penicillium* [Hać--Szymańczuk et al. 2012]. Therefore, the use of oregano as a natural food preservative limiting the growth of food spoilage microorganisms is advisable. Oregano essential oil has been found to have antioxidant activity in meat [Fasseas et al. 2008]. Fresh oregano and its extract were reported to slow down substantially the protein oxidation process and the formation of malonaldehyde in raw pork and beef meat and in ready-to-eat products (sausages and burgers) stored in refrigeration and freezing conditions [Starek and Pawłat 2018]. *O. vulgare* was found to be effective in lowering the oxidation-reduction potential, thus contributing to the maintenance of the natural colour of meat [Wereńska 2013]. Dried oregano leaves are a perfect seasoning for minced and grilled meat. However, 1% oregano oil may introduce a very strong disagreeable flavour to food products, resulting in low sensory quality. Oregano is a strongly aromatic seasoning, and its specific aroma and flavour are ascribed to the content of carvacrol [Zhang et al. 2010].

The leaves and stems of common basil (*Ocimum basilicum* L.) contain volatile oils, plant sterols, flavonoids, tannins, saponins, bitterness, glycosides, and carotenoids [Sadowska et al. 2014]. Its herb contains up to 2.5% of essential oil. The composition of biologically active substances in the oil is dominated by linalool (up to 75%), methylchavicol (up to 87%), eugenol (up to 20%), and smaller amounts of 1,8-cineol and camphor [Wójcik-Stopczyńska and Jakubowska 2018]. Geranial, estragole, epi-α-cadinol, α-bergamotene, α-muurolene have also been detected in basil essential oil [Nabradik and Grata 2016]. The antioxidant activity of basil extracts is mainly associated with the large amount of polyphenolic compounds (phenolic acids and flavonoids) and essential oil, which contribute to reduction of free radicals and inhibition of the activity of oxidizing enzymes in meat and ready-to-eat meat products [Wereńska 2013]. Additionally, hexane and methanol basil extracts have anti-parasitic, antibacterial, and anti-fungal properties (*Candida albicans*, *Streptococcus*, *Aeromonas hydrophila*, *Citrobacter freundii*, *E. coli*, *Hafnia alvei*, and *K. pneumoniae*) [Adigüzel et al. 2005, Wereńska 2013, Nowak 2016, Nabrdalik and Grata 2016]. Dried basil leaves can be used for seasoning any type of meat before further cooking. The common basil has a distinctive strong and spicy-balsamic aroma attributed to the presence of methylchavicol and linalool [Kozłowska-Lewecka and Borowiecka 2011].

Conclusion

Oxidative processes taking place during storage are one of the main causes of deterioration of the quality of meat and its products and pose a serious problem to food technologists. Therefore, it is reasonable to use herbs, which are a rich source of biologically active substances. Polyphenols contained in plants exert a multidirectional effect on food. They exhibit antioxidant activity by stabilization

of fats and other labile food ingredients on the one hand. On the other hand, they function as natural antiseptics limiting bacterial growth and determine the flavour and colour of meat and meat products. These substances, also referred to as bioactive components, are used in the prophylaxis of diet-related diseases. This is particularly important, as they offer a possibility of prevention of the spreading lifestyle diseases.

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Związki biologicznie czynne w ziołach stosowane do mięsa i produktów mięsnych

Streszczenie. Procesy oksydacyjne zachodzące podczas przechowywania i gotowania są jednymi z głównych przyczyn pogorszenia jakości mięsa i jego przetworów, dlatego też stanowią istotny problem dla technologów żywności. Polifenole zawarte w ziołach hamują rozwój niepożądanej mikroflory i ograniczają przemiany oksydacyjne w tłuszczach i białkach oraz decydują o smaku, barwie mięsa i jego przetworów.

Słowa kluczowe: tymianek, rozmaryn, szałwia, majeranek, oregano, bazylia, fenolowe przeciwutleniacze

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Biologically active compounds and pharmacological activity of *Centella asiatica* (L.) Urb.

Summary. *Centella asiatica* herbs have long been used in traditional and folk medicine in China and Southeast Asia as a "panacea" to treat various diseases and skin lesions and to heal wounds. In this study, information about biologically active compounds contained in this species is presented based on literature data. Additionally, the study shows the results of research on the use of extracts from the plant material and their main components in treatment of various types of wounds. The mechanisms of their *in vitro* and *in vivo* action are proposed as well. The healing effect of the pennywort is compared with that of commonly used commercial products. The phyocompounds contained in the Asiatic pennywort have also been shown to have neuroprotective activity, e.g. antioxidant effects, protection of the mitochondrial complex, and prevention of neurodegenerative brain disorders.

Key words: Asiatic pennywort, neuroprotective effect, phytocompounds, phytotherapy, wound healing

Introduction

The Asiatic pennywort (*Centella asiatica* (L.) Urb.) is a medicinal plant from the family *Apiaceae* (*Umbeliferae*), subfamily *Mackinlayoideae* [Liu et al. 2003]. The species has long been used in folk medicine in China and Southeast Asia as a "panacea" for treatment of various skin diseases and as a wound-healing agent [Wu et al. 2012]. The *C. asiatica* herb is the herbal raw material (*Centellae asiaticae herba*). The Asiatic pennywort is an unusual source of various groups of biologically active substances with a wide range of pharmacological activity, which are widely used in supportive treatment of various conditions [Soumyanath et al. 2012, Bylka et al. 2014, Hamid et al. 2016]. To sum up, their therapeutic properties are mainly attributed to the phenolic components

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[Zainol et al. 2003]. Given its content of various biologically active substances, the species has been used in the pharmaceutical [Mala and Tulika 2015, Roy and Bharadvaja 2017], cosmetic [Singh-Verma 2002], and food industries as a food additive [Hashim et al. 2011].

The aim of the study was to present bioactive substances identified in *Centella asiatica* (L.) Urb. and the current knowledge of their wound healing effect and neuroprotective activity reported in scientific literature.

Biologically active substances

Centella asiatica is a source of a wide range of bioactive substances used in phytotherapy. One of the main groups of compounds present in this species comprises pentacyclic triterpenoids, which are divided into oleanae- and ursane-type, depending on the methyl substituent in the C19 and C20 backbones (with double bonds between C12-C13, C13-C18, and C20-C21) [Matsuda et al. 2001a, James and Dubery 2009, Shao et al. 2014].

The ursane type is represented by e.g. asiaticoside C, D, E, F [Jiang et al. 2005] and isothankunic acid [Dutta and Basu 1968], whereas the oleanae group comprises terminolic acid and centellasaponin D and A [Matsuda et al. 2001a, Schaneberg et al. 2003]. Besides monoterpenes, sesquiterpenes, and triterpenoids [Rattanakom 2015], the most important active derivatives of the terpenoid group are saponins (madecassoside and asiaticoside) and sapogenins (madecassic and asiatic acids) as well as other pentacyclic triterpenic acids [Matsuda et al. 2001b].

In addition to terpenoids, *C. asiatica* also contains phenolic compounds. One of the groups of this class of compounds are flavonoids (quercetin, kaempferol, catechin, rutin, apigenin, naringin) [Das and Pereira 1990, Zainol et al. 2003, Marinova et al. 2005], which exhibit antioxidant activity [Rice-Evans et al. 1996] and prevent oxidation of LDL cholesterol [Meyer et al. 1998]. Other active phenols are represented by e.g. tannins (phlobatannin) [Krishnaiah et al. 2009, Udoh et al. 2012] and phenolic acids (rosmarinic acid, chlorogenic acid, and isochlorogenic acid) [Orhan 2012, Amilah et al. 2019].

Other biologically important compounds include essential oils containing monoterpenoids, oxidised monoterpenoids, sesquiterpenoids, and oxidised sesquiterpenoids. The most important compounds in this group are α-humulene, β-caryophyllene, bicyclogermacrene, germacrene B/D, myrcene, trans β-farnesene, and p-cymol [Oyedeji and Afolayan 2005].

Pharmacological activity

It has been documented that the bioactive chemical compounds contained in *C. asiatica* exert antimicrobial effects [Idris and Nadzir 2017], i.e. antiviral [Yoosook et al. 2000], antibacterial [Rattanakom and Yasurin 2015a, 2015b], and antifungal activities [Rakotoniriana 2012].

Phytocompounds extracted from the Asiatic pennywort exhibit high antioxidant [Rahman et al. 2013, Thoo et al. 2013], anti-stress [Rao 2013], anxiolytic [Bharathi et al. 2009], and anticancer activities [Hamid et al. 2016].

Various analyses of the bioactive chemical compounds present in the Asiatic pennywort have revealed their immunomodulatory [Siddiqui et al. 2008], hepatoprotective [Sivakumar et al. 2018], and neuroprotective effects [Orhan 2012, Tripathi et al. 2015] as well as anti-inflammatory [Saha et al. 2013], antiulcerative [Abdulla et al. 2010], and healing activity [Ruszymah et al. 2012, Somboonwong et al. 2012, Wu et al. 2012].

C. asiatica phytocompounds have been administered as part of the phytotherapy of Alzheimer's disease [Soumyanath et al. 2012]. Additionally, they are applied as supportive treatment of periodontal diseases [Sastravaha et al. 2005] and can be used in dermatology [Bylka et al. 2014].

Healing effect

The properties of the pennywort in wound healing were known as early as in ancient times in Asia. This is evidenced by an Indian legend of a wounded tiger, which rolled in the leaves of this plant every day. Finally, the animal fully recovered after a few days; hence, the common name of the plant: the 'tiger grass' [Nguyen and Nguyen 2008].

Centella asiatica raw material contains various bioactive compounds with pharmacological effects, e.g. with its content of triterpenoids, mainly glycosides (asiaticoside and madecassoside) and aglycones (asiatic and madecassic acids), it accelerates wound healing [Wu et al. 2012]. Studies on wound healing in rats after oral and topical administration of *C. asiatica* alcohol extract have demonstrated increased cell proliferation and collagen synthesis in the wounding area. An increase in DNA, protein, and collagen contents in granulation tissues, faster and more efficient maturation and cross-linking of collagen, and high stability of this protein were reported. Wounds treated with the extract were found to contract and produce the epithelium faster than control wounds [Suguna et al. 1996].

Asiaticosid extracted from *C. asiatica* was reported to promote angiogenesis in a chicken vascular membrane model. Topical application of 0.2% and 0.4% asiaticosid solutions onto wounds in guinea pigs and diabetic rats, respectively, increased the hydroxyproline and collagen content, increased collagen

tensile strength, and accelerated epithelium production, thus facilitating wound healing [Shukla et al. 1999].

Similarly, in another experiment conducted on rats, *C. asiatica* leaf extracts enhanced wound healing and successfully overcame the inhibitory effect of dexamethasone in the process [Shetty et al. 2006]. In turn, other researchers documented that oral administration of madecassoside at a dose of 6, 12, and 24 mg/kg resulted in almost complete healing of wounds after 20 days in a group receiving the highest dose (24 mg/kg). Histopathological examinations showed that the phytocompound alleviated inflammatory cell infiltration, accelerated wound contraction associated with proliferation of skin fibroblasts, and reduced the levels of nitric oxide and malondialdehyde with a simultaneous increase in glutathione and hydroxyproline content in burned skin cells. Additionally, madecassoside stimulated skin angiogenesis (*in vivo*) and promoted the growth of endothelial cells in a rat aortic ring assay (*in vitro*). Madecassoside was found to exhibit considerable wound healing efficiency and potential to be applied in treatment of burns. The effect of this compound on wound healing may be based on several mechanisms, e.g. antioxidant activity, collagen synthesis, and angiogenesis [Liu et al. 2008].

After a week of application, hexane, ethyl acetate, methanol, and aqueous extracts applied topically once a day on cut wounds and burns in rats increased the tensile strength of wounds in all groups receiving the extracts. In turn, on the 14th day after the burn, fully developed epithelium and keratinisation were found in all groups treated with the extracts. The phytocompounds identified in the extracts included β-sitosterol, asiatic acid, asiaticoside, and madecassoside. The solutions promoted the healing of cut and burn wounds. Asiatic acid present in the ethyl acetate extract had the most effective wound healing activity [Somboonwong et al. 2012]. In turn, Ruszymah et al. [2012] reported that supplementation of low concentrations of aqueous *C. asiatica* extracts in an *in vitro* wound healing model accelerated healing of corneal epithelium wounds.

In vitro and *in vivo* studies assessing the wound healing rate and efficiency as well as the levels of TGF-β1 (transforming growth factor β1) in skin tissues demonstrated that madecassoside and asiaticoside were the major compounds responsible for this process. The former compound was found to be more effective than the latter in the synthesis of type III procollagen (*in vitro*) and in the rate of wound healing (*in vivo*) [Wu et al. 2012].

An asiaticoside-rich fraction obtained from ethanol extracts of the aerial parts of *C. asiatica* exerted a significant effect on human fibroblasts (HDF) and human skin keratinocytes HaCaT (*in vitro*). Topical application of the fraction at a concentration of 2.5%, 10%, and 40% (w/w) substantially accelerated wound healing in rabbits (*in vivo*). Asiaticoside was therefore regarded as a compound involved in wound healing [Azis et al. 2017].

C. asiatica – containing dressing materials

In vitro studies conducted in a rat model showed that a hydrocolloid sodium alginate dressing with the addition of *C. asiatica* provided excellent mechanical properties. Therefore, it can be successfully used for healing of various types of wounds: abrasions, cuts, or infected wounds. This dressing was substantially more effective than commercial products. It was demonstrated that petroleum hydrocarbon resin (PHR) largely improved the mechanical properties of the dressing, e.g. its tensile strength and elongation at break. Highly promising mechanical and healing properties were determined in the case of a dressing consisting of pennywort extract/polyisobutylene/styrene-isoprene-styrene copolymer (SIS)/PHR/liquid paraffin/sodium alginate/croscarmellose sodium in a 1/8/25/25/12/27/2 weight ratio [Jin et al. 2015].

Literature data indicate a beneficial pharmacological effect of *C. asiatica* in wound healing. It is associated with e.g. enhancement of fibroblast proliferation and collagen synthesis as well as antibacterial activity. Gelatine nanofibres supplemented with pennywort extracts were found to exhibit dermal wound-healing activity in a rat model, and the use of electrospun gelatine membranes containing *C. asiatica* (EGC) was much more effective than treatment with gauze, neat gelatine membranes, and commercial wound dressings, as confirmed by histopathological examinations. Additionally, the pennywort extract did not significantly influence the hydrophilicity of the EGC membranes. An additional advantage is the biodegradability of EGC membranes, which are therefore promising materials for treatment of skin wounds [Yao et al. 2017]

Neuroprotective activity

Among the bioactive chemical compounds isolated from *Centellae asiaticae herba*, triterpene compounds were found to influence positively the function of the hypothalamic-pituitary-adrenal system through an increase in the level of monoamine in the brain of rats accompanied by reduction of the amount of corticosterone in their serum [Chen et al. 2005]. Moreover, triterpene saponosides (asiatic acid, madecassic acid) and heterosides (siaticoside, madecassoside) delayed the process of brain cell aging [Orhan 2006]. An aqueous extract of pennywort was reported to inhibit phospholipidase A_2(PLA_2) in neuropsychiatric diseases. Heterosides present in the extract, mainly asiaticoside, inhibited the activity of calciumin-dependent phospholipase A2 (iPLA2), cytosolic phospholipase A2 cPLA2, and secretory phospholipases A2 sPLA2 in primary cultures of rat cortical neurons [Barbosa et al. 2008, Defillipo et al. 2012].

By penetration of the blood-brain barrier, asiatic acid extracted from *C. asiatica* exerted antioxidant and neuroprotective effects [Rather et al. 2018]. Concurrently, it mitigated mitochondrial damage caused by transient ischaemic attack (TIA)

[Krishnamurthy et al. 2009]. As demonstrated by Teerapattarakan et al. [2018], the neuroprotective effect of *Centella asiatica* ECa233 extract in rotenone-induced parkinsonism rats was mediated through the protection of mitochondrial complex I activity, the effects of antioxidants (superoxide dismutase SOD and catalase), and the enhancement of antioxidant enzyme expression. The neurotropic effects of *C. asiatica* include increased dendritic arborization and synaptogenesis, and may be due to modulations of signal transduction pathways such as ERK1/2 and Akt [Wanakhachornkrai et al. 2013].

The neuroprotective effect of *C. asiatica* leaf extract may result from the increased cyclic phosphorylation of adenosine monophosphoric acid. This acid bound CREB (Cyclic AMP response element Winding) proteins in neuroblastoma cells and Aβ (1-42) amyloid present in amyloid plaques e.g. in Alzheimer's disease patients [Xu et al. 2008]. It was found that the Asiatic Pennywort extract showed potential against butyrylcholinesterase and tyrosinase activity. The tyrosinase enzyme plays an important role in the formation of neuromelanin in the brain causing dopamine-depleted neurotoxicity associated with Parkinson's disease [Carballo-Carbajal et al. 2019].

The accumulation of Al^{3+} cations (a ubiquitous toxic element) in the brain is associated with many neuropathological conditions, including Alzheimer's disease [Thenmozhi et al. 2018]. Asiatic acid applied in this disease exerted a neuroprotective effect, reducing the concentration of Al^{3+} cations [Rather et al. 2018] through the $AlCl_3$ chelation function [Salim et al. 2013]. Asiatic acid was reported to a positive effect on memory and learning ability, especially upon continuous administration [Nasir et al. 2011, Prakash and Kumar 2013]. There was also an increase in the length of dendrites and neuronal branching points in centres responsible for these functions in rats [Rao et al. 2012]. It can be assumed that the bioactive chemical compounds contained in the Asiatic pennywort may prevent neuronal damage [Orhan and Kartal 2010].

Conclusion

Many literature reports confirm the beneficial effects of *C. asiatica* extracts and their main components on the wound healing process. Phytochemicals contained in the extracts, i.e. triterpene compounds, including mainly glycosides (asiaticoside and madecasosside) and aglycones (asiatic and madecassic acids), have been found to exert pharmacological activity resulting in e.g. acceleration of wound healing. The promising research results suggest that pennywort extracts and their main components can potentially be used for treatment of skin wounds. The healing effect of *C. asiatica* is reflected in e.g. enhancement of fibroblast proliferation and collagen synthesis as well as antibacterial activity. Among the phytocompounds mentioned above, asiatic acid was found to exert neuroprotective and antioxidative effects. It also mitigated mitochondrial damage

and had a positive impact on memory and cognitive abilities. Further investigations focused on identification of bioactive chemical compounds with a neuroprotective effect and on elucidation of the mechanism of their action are advisable.

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Substancje biologicznie czynne i działanie farmakologiczne *Centella asiatica* (L) Urb.

Streszczenie. *Centella asiatica* herbs od dawien dawna stosowano w medycynie tradycyjnej i ludowej w Chinach i Azji Południowo-Wschodniej jako ‘panaceum’ w leczeniu wielu różnych schorzeń, w tym zmian skórnych, oraz gojeniu ran. na podstawie zebranych danych literaturowych przedstawiono informacje o związkach biologicznie czynnych występujących u tego gatunku. Omówiono rezultaty badań dotyczące zastosowania ekstraktów i ich głównych komponentów w gojeniu różnego rodzaju ran wraz z sugerowanymi mechanizmami działania w modelach badań *in vitro* i *in vivo*. Gojące działanie wąkroty rozpatrywano w zestawieniu z powszechnie stosowanymi produktami komercyjnymi. Wykazano także neuroprotekcyjną aktywność fitozwiązków wąkroty azjatyckiej m.in. antyoksydacyjną, ochronną dla mitochondrialnego kompleksu i zaburzeń neurodegeneracyjnych mózgu.

Słowa kluczowe: wąkrota azjatycka, działanie neuroprotekcyjne, fitozwiązki, fitoterapia, gojenie ran

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Computed tomography and magnetic resonance imaging as potential tools for assessment of the action of diet ingredients and medicinal herbs in the supporting treatment of fatty liver

Summary. The results of many scientific studies and clinical practice have confirmed the diagnostic capabilities of computed tomography and magnetic resonance in imaging of fatty liver. This review compiles information from the literature in the field of dietetics, herbal medicine and diagnostic imaging to show the use of computed tomography and magnetic resonance imaging for assessment of the action of diet ingredients and medicinal herbs in the supporting treatment of fatty liver, giving an outline of this issue for further detailed methodological considerations. The current state of knowledge gives grounds to claim that the considered methods of liver examination may be a tool for valorization of efforts made in the field of dietetics and phytotherapy.

Key words: liver steatosis, phytotherapy, Mediterranean diet, treatment control, diagnostic imaging

Introduction

Fatty liver is caused by abnormal metabolism of lipids, which accumulate as droplets inside the liver cells. Steatosis of liver cells is the result of their aging, comorbidities, toxic influence of drugs or an unfavourable lifestyle [Bradbury 2006 – cited by Dai et al. 2014, Bellentani and Marino 2009, Donato and Gómez-Lechón 2012, Levelt et al. 2016, Ogrodnik et al. 2017]. Untreated, even a mild form of this disease may lead after some time to such serious diseases as

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steatohepatitis, fibrosis, cirrhosis, and even liver cancer [Hashimoto et al. 2009, Ascha et al. 2010 – cited by Dai et al. 2014]. Persons with alcohol-induced steatosis have a risk of alcoholic steatohepatitis (ASH), hepatic fibrosis, cirrhosis and hepatocellular carcinoma [Donohue 2007]. Non-alcoholic fatty liver disease (NAFLD) is a result of fat accumulation in the liver (liver fat >5–10% of liver weight), which is not related to excess alcohol consumption or other causes of steatosis, while its severity ranges from simple hepatic steatosis or fatty liver to non-alcoholic steatohepatitis (NASH) [Kontogianni et al. 2014].

Pathogenesis and possible supporting treatment with herbs

One of the factors causing this disorder is carbon tetrachloride (CCl_4) metabolized mainly in the liver. Liver damage can occur after transformation of CCl_4 to free radicals, e.g. trichloromethyl radical (CCl3*) and its derivative trichloromethylperoxy radical (CCl3OO*), whose formation is catalyzed by the CYP2E1 enzyme [Lewis et al. 2005 – cited by Dai et al. 2014, Pan et al. 2007].

Proanthocyanidins contained in grape seeds inhibit this process by inhibiting the toxic effects of CCl_4, reducing oxidative stress, and inhibiting the activity of the CYP2E1 enzyme, and thus prevent liver damage. Ingesting proanthocyanidin-containing foods or proanthocyanidin supplements can help to treat both alcoholic and non-alcoholic fatty liver disease by suppressing the damaging liver peroxidation mechanisms associated with steatosis and, therefore, delay or prevent steatohepatitis, fibrosis, cirrhosis, and liver cancer [Dai et al. 2014]. In addition, after testing a dozen species for their therapeutic effects on damage caused by CCl_4 it turned out that *Chrysanthemum balsamita* (L.) Baill., *Echinacea pallida* (L.) Moench., *Calendula officinalis* L. (fluid extracts obtained from dried powdered material by repercolation with 70°-ethanol) and *Corylus avellana* L. contributed to reduction in the intensity of hepatic cytolysis and fatty liver, and restored the proper functioning of some enzymes [Rusu et al. 2005]. Another species (*Curcuma longa* L.), whose active ingredient is curcumin, reduces the fat content in the liver by 78.9%. There were also significant reductions in total serum cholesterol, a decrease in the body mass index, and a decrease in low-density lipoproteins, cholesterol, triglycerides, aspartate aminotransferase, alanine aminotransferase, glucose, and glycated haemoglobin compared to placebo. Curcumin is an effective polyphenol in inhibiting the development of fatty liver and steatohepatitis, although this has not yet been clinically confirmed [Rahmani et al. 2016]. A plant gum extract from *Ferula asafoetida* L. has been found to exert significant anti-obesity, fat lowering, and liver steatosis protective effects in type 2 diabetic rats [Myers et al. 2010 – cited by Azizian et al. 2012]. *Ferula asafoetida* L. gum can be a good candidate for the treatment of type 2 diabetes-induced obesity and hepatosteatosis [Azizian et al. 2012].

Possible influence of diet on the disease

The Mediterranean diet can be used to reduce the occurrence of fatty liver. It consists of fruits, vegetables, unrefined grains, potatoes, moderate consumption of fish and its derivatives, and low consumption of meals with large amounts of fat, red meat and derivatives [Zelber-Sagi et al. 2007 – cited by Kontogianni et al. 2014]. In experiments with rats with non-alcoholic fatty liver disease, moderate consumption of olive oil was found to reduce the accumulation of triglycerides in the liver of the rats [Hussein et al. 2007 – cited by Kontogianni et al. 2014]. During *in vivo* experiments, this oil prevented fibrosis, compared to food with polyunsaturated fatty acids [Szende et al. 1994, Kontogianni et al. 2014]. The main phenolic compound in olive oil, oleuropein, reduced fatty liver in mice fed a high-fat diet [Park et al. 2011 – cited by Kontogianni et al. 2014]. A diet should be rich in monounsaturated fatty acids and omega-3 fatty acids, fruits, vegetables, and fibre. It should be characterized by reduced amounts of saturated fats, simple carbohydrates, sweetened drinks and alcohol [Abenavoli et al 2014]. Although the Mediterranean diet is not associated with a lower likelihood of development of NAFLD, it is associated with a lower degree of insulin resistance and less severe liver disease in patients with NAFLD [Kontogianni et al. 2014].

Assessment of the possible supporting treatment using CT and MRI

How to observe the influence of the methods described above on the intensity of hepatic steatosis without interventional procedures? The results of studies published in subsequent years prove that various techniques can be used in the diagnosis and quantification of fatty liver in its various forms including focal and multifocal hepatic steatosis. Each of the following techniques requires separate characteristics: ultrasonography, ultrasound-based elastography, computed tomography (CT), magnetic resonance imaging (MR or MRI), MR spectroscopy, intravoxel incoherent motion diffusion-weighted MR imaging, positron emission tomography–computed tomography (PET/CT), and vibration controlled transient elastography [Noworolski et al. 2012, Keramida et al. 2014, Lee and Kim 2015, Tang et al. 2015, França et al. 2017, Yoneda et al. 2018, Zheng et al. 2017, Nejabat et al. 2018, Wang et al. 2018, Obmann et al. 2019, Siddiqui et al. 2019]. Focusing on the possibilities of computed tomography and magnetic resonance imaging, it is worth paying attention to the results of research from the last several years. As demonstrated by Graffy et al. [2019], comparison of the CT fat fraction in a specific patient with population norms is possible based on liver Hounsfield unit measurement with manually selected region of-interest (ROI) or by an automated algorithm for CT-based liver segmentation and attenuation assessment. Moreover, liver and spleen attenuation indices in CT examinations

have higher performance in diagnostics of hepatic steatosis than indices from liver attenuation alone [Byun et al. 2019]. Standardization of the method allows this technique to diagnose the condition of the liver and facilitates decisions about treatment or participation of potential donors in transplantation. As reported by Adalı et al. [2019], liver biopsy could be avoided in donors with BMI <30 kg/m^2 and a computed tomography liver attenuation index > 6, but the diagnostic accuracy of computed tomography for predicting hepatic steatosis decreases with increasing BMI. Similarly, a combination of MR fat quantification and MR elastography can provide sufficient sensitivity to detect liver steatosis or fibrosis in candidates for liver donation [Yoon et al. 2015].

Nowadays, MRI is a main technique in medical imaging, including monitoring the treatment progress [Meisamy et al. 2011, Cui et al. 2016, Alsina et al. 2017]. It has also become a good tool for scientific studies on effects of various factors (e.g. pharmacological agents and harmful environmental factors) on living organisms [Gonet 1997]. Examination with this technique allows qualitative and quantitative assessment of fat in the liver (e.g combined compressed sensing and parallel imaging and multi-echo chemical shift-encoded gradient echomagnetic resonance imaging) [Mann et al. 2016, Lee et al. 2020]. Advanced fibrosis in NAFLD can be detected using MR elastography [Kim et al. 2013, Schwimmer et al. 2017], but it is important to underline that MR elastography may be useful in early detection of steatohepatitis appearing in a stage before fibrosis [Salameh et al. 2009]. As demonstrated by França et al. [2017] multi-echo chemical shift-encoded gradient echomagnetic resonance (MECSE-MR) sequence simultaneously quantifies liver steatosis and siderosis, regardless of coexisting liver inflammation or fibrosis, with high accuracy in a wide spectrum of diffuse liver disorders. The results of a study conducted by Evers et al. [2015] show that diffuse reflectance spectroscopy can quantify steatosis in liver tissue both *in vivo* and *ex vivo* with good agreement compared to histopathology analysis and can be performed in real time and be useful in clinical practice. It is important to take into account the coexistence of fibrosis and steatosis of the liver, for example in patients with primary biliary cirrhosis. As suggested by Friedrich-Rust et al. [2010], MR-spectroscopy is the best method for measurement of liver steatosis and the diagnosis of this disorder is correlated with histologic steatosis. However, it must be added that it is important to consider the iron content of the liver [Sharma et al. 2014].

Final comments

The usefulness of imaging techniques in monitoring treatment and evaluation of new drugs or their potential harmful effects is being investigated [Guaraldi et al. 2012, Jayakumar et al. 2019]. In addition to determination of the suitability and precision of the method for assessment of the effects of a specified drug, it

is necessary to maintain and control its quality, since the pharmaceutical industry is interested in a strictly standardized drug, as underlined by Dębska [1983]. The diagnostic methods described above may be useful for diagnosis of the occurrence, intensity, and evolution of hepatic steatosis. It can also be used in both clinical practice and research, e.g. CT indices for liver and spleen attenuations analysed in a study by Byun et al. [2019]. Observations of liver can play an important role in monitoring chronic rare diseases like Prader-Willi syndrome [Mele et al. 2017].

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Tomografia komputerowa i rezonans magnetyczny potencjalnie użyteczne do oceny działania składników diety oraz ziół leczniczych we wspomagającym leczeniu stłuszczenia wątroby

Streszczenie. Wyniki wielu badań naukowych i praktyka kliniczna potwierdziły możliwości diagnostyczne tomografii komputerowej i rezonansu magnetycznego w obrazowaniu stłuszczenia wątroby. Niniejsze opracowanie zawiera przegląd literatury z zakresu dietetyki, ziołolecznictwa i diagnostyki obrazowej w celu przedstawienia, w jaki sposób obrazowanie technikami tomografii komputerowej i rezonansu magnetycznego może być stosowane w ocenie działania składników diety i ziół leczniczych we wspomagającym leczeniu stłuszczenia wątroby, oraz dostarcza zarys problematyki do dalszych szczegółowych rozważań metodologicznych. Obecny stan wiedzy daje podstawy do tego, by stwierdzić, że rozpatrywane sposoby badania wątroby mogą stanowić narzędzie do waloryzacji wysiłków podejmowanych w zakresie dietetyki i fitoterapii.

Słowa kluczowe: stłuszczenie wątroby, fitoterapia, dieta śródziemnomorska, kontrola leczenia, diagnostyka obrazowa