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Arctium lappa - A POTENTIAL SOURCE OF BIOACTIVE COMPOUNDS WITH PHARMACEUTICAL APPLICATIONS

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Abstract

The study provides an overview of the bioactive molecules present in Arctium lappa. The main bioactive compounds in this plant, their pharmacological activities, and the main methods of obtaining bioproducts concentrated in these bioactive compounds are summarized. Due to the diversity of pharmaceutical activities of the bioactive components found in Arctium lappa (biomolecules with anti-inflammatory, antimicrobial, antioxidant, antitumor properties) this species can be considered a potential source of compounds with therapeutic properties of interest.

Key words*: Arctium lappa, pharmacological activities, bioproducts.*

INTRODUCTION

Arctium lappa (*Asteraceae*) known as burdock (Jiang et al., 2019), is a biennial plant that grows to about one meter in height, blooms from July to October, and is widespread and cultivated in East Asia and Europe (Skowronska et al., 2020). *Arctium lappa* can also be grown at temperatures between 10 and 25°C, but shows better development between 16 and 22°C, on moist and sandy soils (Skowronska et al., 2021; Tita et al., 2009).

In terms of chemical composition, the major active chemical compound, distinctive for *Arctium lappa*, is arctigenin (AR) and its glucosylated form arctiin, a phenolic compound of the lignan class (so it is part of lignin); arctigenin and arctiin are in the category of plant estrogens or phytoestrogens, due to their dibenzylbutanic backbone (Gao et al., 2018). Arctigenin and arctiin were identified in the fruits of *Arctium lappa*, from which is derived the plant name "arctii", and is widely used in China and other countries due to its biological activity (Chen et al., 2016).

The major secondary metabolites of the species are tannin and iron complexes, polyacetylenes, sulfuric acetylenes, essential oils, guainolides, bitter compounds, lignans (arctigenin, arcthin), and sterols (sitosterol, stigmasterol) (Maghsoumi et al., 2016). Studies on the metabolic profile of the compounds present in various parts of *Arctium lappa* have also shown the presence of polyphenols (4-o-glucoside caffeic acid, chlorogenic acid, quercitrin, quercetin, quercetin-3-O-glucuronide, nobiletin, p-coumaric acid, biachanin A, and tangeretin), tannins, and terpenoids (lupeol, ursolic acid, oleanolic acids) (Al-Snafi, 2014), gallic acid (Nema et al., 2019) but also some polysaccharides as inulin and pectin (Watanabe et al., 2020). Thus, according to the literature, burdock root, *Bardanae radix*, is used as a medicine, but also as a vegetable (similar to potatoes) in East Asian regions due to its respective nutritional value of high carbohydrate content (69%), inulin (27-50%), mucilages, to which fats, vitamins (B1-B6, C; E; K) and minerals (Ca; Fe; Mg; P; Zn) is added (Gentil et al. 2006; Awale et al. 2006).

Regarding secondary metabolites of burdock, studies have shown that the pharmacological activities are due to the rich content of caffeoylquinic derivatives, and flavonoids such as rutin, hyperoside, isoquercitrin, and quercitrin (Liu et al. 1997). According to literature, when traditional medicine is concerned, burdock root is used in skin conditions, kidney and liver disease, cancer, and diabetes (Maghsoumi et al., 2019). The leaves of this species have a high content of polyphenols, (Kim et al., 2020) which are used as treatments for burns, ulcers, and wounds (Carlotto et al., 2015). The seeds of Burdock (called niubangzi in China) are used in Chinese medicine and have anti-inflammatory, detoxifying, diuretic, sedative, and anti-inflammatory properties (Qina et al., 2019). Moreover, *Arctium lappa* is effective as a treatment for inflammatory diseases, raised tension, or viral hepatitis (Leea et al., 2019).
In Romanian popular medicine, the extract obtained in hot water (decoction) is used to prevent or relieve cough, being thus recommended for various lung, digestive, renal, and skin diseases (Maghsoumi et al., 2016; Pereira et al., 2005). Therapeutic applications were attributed to different parts of the plant, roots, leaves, seeds, and fruits. All of these are used to obtain bioproducts used in the treatment of intoxications, throat infections, rashes, and skin infections (Carlotto et. al., 2015).

METHODS FOR OBTAINING ENRICHED FRACTIONS IN BIOMOLECULES OF INTEREST

Regarding the location of the active compounds in the parts of the plant, lignans were identified in all parts of the plant. Arctigenin is present in higher concentrations in leaves, fruits, seeds, and roots (Table 1); actiin is found in leaves, fruits, and roots; diarctigenin is found mainly in fruits, roots, and seeds (Al-Snafi et al., 2014). Trachelogenin is mainly extracted from fruits; lappaol F is extracted from fruits and seeds; terpenoids such as beta-eudesmol and 3α-hydroxylanosta-5 have been isolated mainly from fruit. Regarding polyphenols, caffeic acid is present in large quantities in stems, leaves, and roots. Chlorogenic acid is dominant in leaves and roots and the tannins, inulin, and sterols are found in large quantities in the roots. Burdock root is considered an important source of fiber (prebiotic), cynarin, lignans, and quercetin (Al-Snafi, et al., 2014). The extraction methods used depend on the solubility of the compounds from the part of the plant used. For example, in the case of *A. lappa* root (Agha et al., 2020), lignans, fructans (polysaccharide fraction), polyphenols, amino acids, and phytosterols are compounds of maximum interest. The method of extraction of the secondary metabolites from the root is carried out with alcohol of different concentrations, more specifically with ethanol or methanol solutions (Machado et al., 2012). Regarding the proof of pharmacological activity of root extracts, it was reported that both ethanolic (EtOH) and ethyl acetate (EtOAc) extract inhibits the CaCo-2 cell lines proliferation. Antiproliferative activity was progressively improved by subsequent extraction in n-hexane (EHX). The efficacy of EHX extract was superior in comparison with bioproducts obtained in other solvents, for MCF-7 and EAhy926 cell lines. For these cell lines, the IC50 values obtained were 14.08±3.64 and 27.25±3.45 μg/ml, respectively (Machado et al., 2012). The EHX fraction also significantly regulated TGF β cytokine values in MCF-7. The efficacy of EHX against the MCF-7 adenocarcinoma cell line consisted in the regulation of the transcription factor NF-κB, which has a role in blocking cellular apoptosis (Machado et al. 2012). In a study performed on the leaves of *A. lappa*, other researchers showed that the extracts obtained in ethanol show high antioxidant activity in vitro. In a recent study, Jiang et al. evaluated the antioxidant capacity of the mono-, di- and tri-CQA (one to three units of cofeyl-bound quinidine, respectively), isolated in the alcoholic extract made from burdock root, using DPPH and FRAP methods (Jiang et al., 2016). Inulin, present in large quantities in burdock root (17%), belongs to the category of low molecular weight fructans (Liu et al., 2014). Studies performed on three fractions, named ALP40-1, ALP60-1, and ALP80-1, with an average molecular weight of 218 kDa 178 kDa, and 60 kDa, respectively, proven that these have a higher glucose content than fructose. Taleb and coworkers revealed that the fraction ALP60-1 has the best

antioxidant activity (Agha et al., 2020). Experimental studies made to elucidate this behavior have shown that the pronounced antioxidant activity of a polysaccharide obtained from *P. tricuspidata* (named PTP-4) is possible to be due to its structure's mannose content (Liang et al., 2018).

Studies performed on a fructan (named ALP1) with a molecular weight of about 4600 Da obtained from the roots of *A. lappa* using extraction with hot water revealed a significant antioxidant activity both *in vitro* and *in vivo* (Lou et al., 2009).

Studies performed to evaluate the IC50 for the antioxidant activities of ALP60-1 for two strong radicals (superoxide and hydroxyl) indicated values of 0.79 mg/ml and 1.38 mg/ml, respectively.

An optimized ultrasonic extraction method (83 min) was used to obtain ALP-60-1 (Liang et al. 2018). The AA activities of ALP60-1 could therefore be attributed either to the high mannose content (5.72%) or to the ratios of the various monosaccharides present in its structure (Liang et al., 2018).

Another process of obtaining fructans (APP) consists of the extraction of dry powder obtained from the roots of *A. lappa* L. with petroleum ether and ethanol for 3 hours. After the removal of the solvents, the solid residues were dried in a vacuum and used for ultrasonic extraction of crude ALP (fructans) (Jiang et al., 2019). The Sevag method was used to eliminate the proteins; the deproteinized solution was dialyzed at 8000 Da; after that, it was concentrated by evaporation. In a concentrated solution, anhydrous ethanol is added so that in the final solution the concentration of C_2H_5OH is 40% (ALP40), 60% (ALP60), and 80% (ALP80). The solutions thus prepared were left overnight at 4°C and then centrifuged at 4500 rpm for 15 min. After centrifugation, each precipitate was washed more times with C_2H_5OH (99%) and acetone and then was lyophilized to obtain a crude extract of ALP40, ALP60, or ALP80 (Jiang et al., 2019).

Concerning the extraction of polyphenols from the *Bardanae radix*, the highest concentrations of phenolic compounds were reported in the extraction of plant material in Soxhlet with solvent CH_2Cl_2 (79.45 mg GAE/g product) and EtOH (77.26 mg GAE/g product) (Predes et al., 2011).

Other scientists found that while the hydroethanolic extract (HE) contains 72.61 mg GAE/g product, the extraction with a mixture of CH_3Cl-C_2H_5OH (1:1), conduce to the content of 85.15±0.55 mg GAE/g dry brut extract.

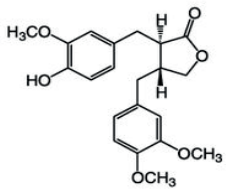
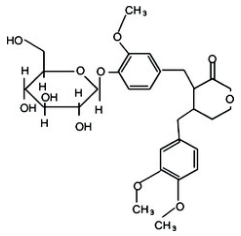
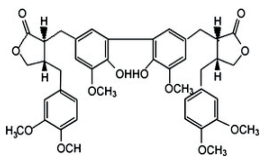
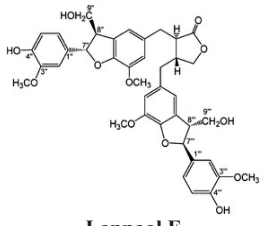
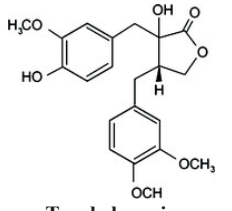
The products obtained with solvent mixtures contain high amounts of flavonoids 12.57±0.05 mg Quercetin/g product (Gilioli et al., 2007).

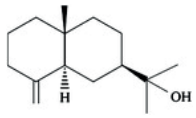
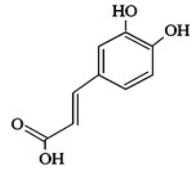
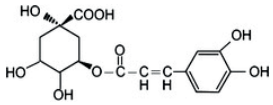
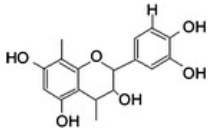
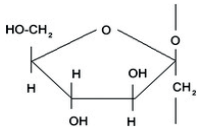
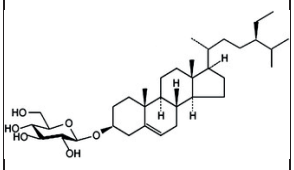
Predes and collab. have reported that in the product obtained by extraction with CH_3Cl the polyphenols content can attain 65.92±0.36 mg GAE/g (Predes et al., 2011).

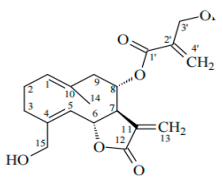
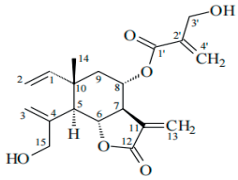
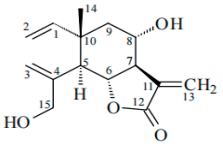
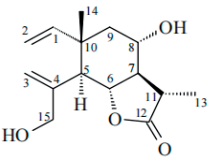
In this context, it has been determined that phenolic compounds such as anthocyanin, carotenoids, and flavonoids are responsible for the strong AA of burdock root extracts (Jianga et al., 2016). Other studies performed on polyphenolic content (Folin-Ciocalteu method) from *Bardanae radix* extracts found the following: the alcoholic extract (70% C_2H_5OH) contains 216.75 mg GAE/g (dry biomass); the C_6H_{14} extract contains 41.58 mg GAE/g; the $CHCl_3$ extract have 69.92 mg GAE/g; the aqueous extract has 96.92 mg GAE/g (Lea et al., 2019). The results obtained from these measurements show the fact that in the alcoholic media (ethanol) the polyphenolic compounds are best extracted (Jianga et al., 2016). The content of polyphenolic compounds from the roots is much lower in comparison with the polyphenols levels from the seed or leaves of the burdock plant (Thaísa et al., 2020).

Regarding the extraction of sesquiterpenes, studies performed on *A. lappa leaves* (Savina et al., 2006) have shown that the best extraction solvent is EtOAc. These extracts contain onopordopicrin and other sesquiterpenes, and their concentration in EtOAc attain 0.035-0.005% and 0.01% respectively (sesquiterpenes).

Table 1. Biomolecules of interest contained in *Arctium lappa*

Compound name/ Chemical structure	Methods of obtaining	Methods of identification	Biological activity	References
 **Arctigenin**	Extraction in 35% C_2H_5OH with reflux; after concentration, selective extractions are performed with petroleum ether, CH_2Cl_2, and C_4H_9OH	HPLC (Ultra performance liquid chromatograph) coupled with mass spectrometry; HR-ESI-MS (high-resolution mass spectrometry) with electrospray ionization); IR; UV-VIS; NMR	Antitumor activity; Antiviral activity; Anti-inflammatory activity. Anti-influenza virus.	Jianga et al., 2016; Zeng et al., 2018; Predes et al. 2011
 **Arctiin**	1.5 ml + 1 ml of 80% CH_3OH (23°C) for 8 hours	LC−MS, MALDI-QIT-TOF MS	Antiprolife-rative activity against B cell hybridoma cell, MH60; Antitumor activity; Antiviral activity	Liu et al., 2014
 **Diarctigenin**	Extraction with CH_2Cl_2, C_2H_5OH (95%) and water (plant solvent ratio: 2: 1) for 6 hours for each solvent	Folin-Ciocalteau method	Antioxidant activity; Anti-inflammatory activity; Inhibiting NO production	Predes et al., 2011
 **Lappaol F**	Extraction by maceration with a 96% C_2H_5OH	UPLC-ESI-QTOF-MS qualitative analysis; GC-MS	Antiviral activity; Antitumor activity	Dias et al., 2017
 **Trachelogenin**	Accelerated solvent extraction (extraction with liquid under PLE pressure) 200 g of dry vegetable material is subjected to PLE extraction for 1.45 h using 550 ml of 70% C_2H_5OH		Antioxidant activity; Antidiabetic activity	Petkova et al., 2020

Compound name/ Chemical structure	Methods of obtaining	Methods of identification	Biological activity	References
 **Beta-eudesmol**	Extraction with supercritical fluids (solvent: CO2; cosolvent: C_2H_5OH) Extract 1 was obtained below 15 MP, in 50 minutes, and extract 2 under 15 MP in 75 min	GC-MS	Anti-inflammatory activity; Antibacterial activity	Chan et al., 2011
 **Caffeic acid**	Extraction by maceration at 45°C for 48 hours with petroleum ether, C_2H_5OH and water (MTT tests). The extract obtained in C_2H_5OH had the highest biologic activity		Antioxidant activity; Antitumoral; Immunomodula -tory activity	Agha et al., 2020
 **Clorogenic acid**	Soxhlet extraction for 18-12 hours. *Arctium lappa* leaves were extracted in 70% C_2H_5OH		Neuroprotecto; antioxidant; anti-HIV	Agha et al., 2020; Chan et al., 2011
 **Tannin**	The alcoholic extract (made in 70% C_2H_5OH) is obtained by refluxing (3 times), at 100°C for 1 hour each, using 200 mL C_2H_5OH and 10 g of plant material		Antioxidant activity; Antitumoral; Immuno-modulatory activity	Chan et al., 2011
 **Inulin**	The dried burdock roots (20 kg) were extracted with 300 L C_2H_5OH at 80°C (3 times), at reflux, for 2 hours. Partition with petroleum ether (V_{water}:$V_{petroleum\ ether}$ = 1:13); dichloromethane (V_{water}: $V_{dichloromethane}$ = 1:1); 3 times and ethyl acetate **(**EtOAc) (V_{water}: V_{EtOAc} = 1:1); 6 times	NMR spectra IR spectra	Prebiotic activity; antihypertensiv; antidiabetic	Gaoa et al., 2020; Chan et al., 2011; Skawronsk a et al., 2021; Jiang et al., 2019. Zhang et al., 2019
 **Sitosterol-beta-D-glucopyranoside**	Maceration with methanol (2 L) at 25°C for 3 days. The extract was concentrated, and fractionated with open column of silica gel (210 g) using gradual elution with $CHCl_3$ and MeOH in various ratios (10:0, 50:1, 10:1, 5:1, 1:1, 1:5, and 0:10) with a volume of 840 ml for each ratio, to give 14 fractions (420 mL/fraction).	Mass spectra NMR analysis performed in $CHCl_3$	Antidiabetic activity.	Skawronsk a et al., 2021; Jiang et al., 2019.

Compound name / Chemical structure	Methods of obtaining	Methods of identification	Biological activity	References
 **Onopordopicrin**	The crude extract obtained in 50% C_2H_5OH was partitioned with C_6H_{14}, EtOAc and n-C_4H_9OH.		Antiproliferative activity	Machado et al., 2012
 **Dehidromeltesina**	Extraction with 70% C_2H_5OH at 40° C for 2 hours (at reflux). The filtered extract is concentrated and fractionated consecutively with n-C_6H_{14}, $CHCl_3$, EtOAc and water	UPLC-Q-TOF MS	Antioxidant activity	Leea et al., 2019
 **Dehydromelitesin**	Treatment of 30 g of ground material with 400 ml of diffrent solvents, choosed in order of increasing polarity: C_6H_{14}, EtOAc and 100% C_2H_5OH	LC-ESI-MS	Antioxidant activity	Ayoddhia et al., 2019
 **Melitesin**	Acetone extraction (plant solvent ratio: 1:20), for 1 h	LC-ESI-MS	Antioxidant activity	Olennikov & Tankhaev et al., 2011

METHODS FOR CHARACTERIZING BIOPRODUCTS CONTAINING MOLECULES OF INTEREST

Studies performed by Lin and Harnly on caffeoyl quinic derivatives in burdock root propose the LC-DAD-ESI/MS as analyzing method. Comparative analytical results on cultured burdock root indicate chlorogenic acid and cinnarine levels are 50% higher than the levels found in the same species from spontaneous flora (Lin. et al. 2008) techniques. In addition to the above-mentioned compounds, this method made it possible to identify 18 other hydroxycinnamoylquinic acids in the form of mono-, di- or tricaffeoylquinic compounds (Lin and Harnly, 2008). Ferracane and coworkers characterized polyphenolic compounds from seeds, root leaves using the LC/MS technique (Ferracane et al., 2010). A UPLC/MS/MS method was developed to identify benzoic acid, p-coumaric acid, and flavonoids from burdock leaves (Table 1) (Lou et al., 2009). Research conducted by other scientists revealed the presence of the luteolin, by using the HPLC-MS technique. The first study on the quantification of lignans in burdock root was conducted by Liu and collab., which used lyophilized roots from six different genotypes of Chinese origin (Liu et al., 2015). This study determined the concentration of arctinine present in the dried root and root bark, which ranged from (20-40) mg/100 g and from (130-210) mg/100 g, respectively. In the case of arctiin and arctigenin, Liu and collab. have indicated the HPLC techniques, as a method to identifying

these compounds (Liu et al. 2014). Predes et al. have used the HR-ESI-MS techniques to determine the presence of quercetin and arctigenin in the hydroethanolic extract from burdock root (Predes et al. 2011). Thus, the concentration of arctigenin (1.27 mg/100 g), caffeic acid (2.18 mg/100 g), chlorogenic acid (0.68 mg/100g) and quercetin (1.82 mg/100 g) were determined. Haghi et al have examined possible differences between cultivated burdock root (BC) and wild root, which grows freely in the wild (BS) (Haghi et al., 2013). The authors used HPLC and UPLC as techniques for the quantitative evaluation of chlorogenic acids. Jiang and collab. highlighted the fact that the extraction of polysaccharides is carried out mainly in an aqueous solvent, (hot water) (Jiang et al., 2019). It has thus been determined that high temperature and long extraction time can degrade polysaccharides, thereby reducing their pharmacological activity. Thus, the ultrasonic-assisted extraction (UAE) variant was proposed, due to the accelerated process, and minimal effects on the structure of the molecules. According to these studies, polysaccharides obtained by the ultrasonic method present an antioxidant activity higher than those obtained by other methods. By applying a combined method (microwave and ultrasonic oven) for extraction of polysaccharides fractions, the extraction time was reduced from 15 min to 1 min (Lou et al 2009). Other authors have used these technologies who have conducted comparative extraction studies for the two processes. The result obtained consisted of increasing the yield of the extraction process of polysaccharides from burdock root from 12% to 24%. Most of the compounds identified were caffeic quinic acids, of which four were mono caffeoylquinic acids, six dicafeoylchinic acids, and two tricafeoylchinic acids. (Milani et al., 2012). Similarly, given that burdock roots are a valuable source of fructooligosaccharides (17%) (Zhang et al., 2018), Milani and collab. have demonstrated the efficiency of the inulin extraction process from burdock roots in the presence of high-intensity ultrasound, with an extraction yield of 12% inulin (Milani et al., 2012). Microwave-assisted extraction has been used for the extraction of fructans, inulin, and ten sugars, all from burdock roots (Liu et al., 2014). The UPLC technique was used to identify the major caffeine compounds from burdock root, 5-CQA, and 1,5-DCQA (esters consisting of one molecule of quinic acid with one or two units of caffeic acid, respectively). These identified compounds exhibit an inhibitory effect on viruses like HSV-1; HSV-2; ADV-3, ADV-11, or HIV-1 (Haghi, et al. 2013; Chiang et al., 2002; Yang et al., 2005).

BIOLOGICAL ACTIVITIES OF BIOMOLECULES OF INTEREST CONTAINED IN *Arctium lappa*

Studies performed have shown that the biological activities of bioproducts derived from burdock are due to lignans, arctiin, arctigenin, and polysaccharides (Table 1). These, in combination with polyphenols (flavonoids and polyphenolcarboxylic acids), exert antitumor, antibacterial, antiviral, hepatoprotective, anti-urolytic activities (Jingvi et al., 2012; Chan et al., 2011).

ANTIMICROBIAL ACTIVITY

Chlorogenic acid isolated from burdock leaves inhibits the development of microorganisms E. coli, *S. aureus*, and *M. luteus* (Lin et al., 2004). Antimicrobial activity exhibits the volatile fractions obtained from leaves and seed of A. lappa, for microorganisms such as *B. subtilis, E. coli, A niger*, and *C. albicans* (Aboutabl et al., 2013). Solid bioproduct obtained from *A. lappa* leaves by lyophilization, inhibits the microorganisms such as *B. subtilis, C. albicans, L. acidophilus,* and *P. aeruginosa* (Pereira et al., 2005; Oliveira et al. 2014). The bioproducts obtained by *A. lappa* by extraction with EtOAc, are useful in treating stomatological infections with *C. albicans, E. coli, L. acidophilus*, *P. aeruginosa*, *S. aureu*s, and *M. luteu*s (Gentil et al., 2006; Tita et al. 2009). *Arctium lappa* root extract inhibits the development of *Klebsiella pneumonia*; the mechanism of action is probably due to the inhibition of β-lactamases (Rajasekharan et al., 2017). Studies have confirmed that burdock bioproducts exhibit antiviral activities against A/NWS/33, H1N1; IFV (arctiin and arctigenin) (Hayashi et. al., 2010); HSV-1; HSV-2; ADV-3; ADV-11; HIV-1 (Matsumoto et al., 2006; Liu and Tang, 1997). A study conducted in 2012 showed that 3.7 billion people (approximately 67% of the

world's population) had HSV-1 infection (Bacon et al., 2003).

ANTIOXIDANT ACTIVITY

In a study performed by Lou and collab., antioxidant capacity (A.A.) was associated with the content of the polyphenolic compound from burdock (Lou et al., 2009). Biomolecules such as chlorogenic acid, o-hydroxybenzoic acid, caffeic acid, p-coumaric acid, and rutin, found in extracts from *A. lappa* leaves, are involved in biological activities as A.A. The bioproducts obtained from burdock leaves are effective in the treatment of some eye disorders (Lee et al., 2020). Studies performed on the polysaccharide fractions isolated from the root of *A. lappa* (called *Bardanae radix*) have shown beneficial effects on patients with diabetes, especially due to their antioxidant effect. In this context, the co-presence of caffeoylquinic derivatives, (chlorogenic acids), responsible for the strong antioxidant activity of burdock root extracts, has been demonstrated (Ravini Ayoddhia et al., 2019). Similarly, starting from the observation that a high level of glucose favors the activation of PKC (protein kinase, enzyme with a role in gene expression and regulation responsible with inflammation and disturbance of lipid metabolism in diabetic lab animals), Liu and collab discovered that the polysaccharides from burdock can influence the PKC activity, modifying the lipidic metabolism in lab animals with induced diabetes (Li et al., 2019). In the context of the benefits of antioxidant compounds, oleamide was identified as the bioactive compound responsible for the antiallergic activity of burdock roots. Studies performed on lab animals were reported attenuation of levels of histamine, TNF-α, and interleukins (Yang et al., 2016; Ayoddhia et al., 2019). The authors conclude that the identification of oleamide may contribute to further research to reduce the allergic response by burdock root administration. Possible therapeutic effects of burdock root have been reported by other scientists (Jiang et al., 2019; Maghsoumi et al., 2016). The hepatoprotective effect of burdock roots, in close connection with the antioxidant effect, has also been reported in two studies, both with similar doses (300 mg/kg body weight) using different extracts. Predes and collab. have studied the effects of the administration of a burdock ethanolic extract in case of hepatotoxicity generated by the administration of cadmium and reported the complete restoration of biochemical functions. Previous studies performed on lab animals with paracetamol-induced liver damage (800 mg/kg in rats) showed that in administering a burdock extract, normalization of liver function in rats is achieved after 30 days. Both results indicated that burdock roots contributed to the recovery of liver function (Predes et al., 2014). The antioxidant effect has been proven in studies performed by Tian and collab. who investigated the possible neuroprotective activity of burdock root extract in human neuroblastoma affected by H_2O_2. Increased cell viability was observed for burdock extract at concentrations of 40-80 µg/ml. The antioxidant capacity and antiapoptotic activity of burdock extract have been associated with the neuroprotective effect (Tian et al., 2015).

THE MECHANISM OF ACTION OF THE MOLECULES OF INTEREST CONTAINED IN *Arctium lappa*

The active biomolecules of *A. lappa* are arctiin; arctigenin; caffeic acid; chlorogenic acid; cinnarine; benzoic acid; p-coumaric acid; rutin; quercitrin; quercetin and luteolin. All these, together or separately, are associated with the medicinal properties of this species (Thaisa et al., 2020). As previously mentioned, the major active chemical compound, distinctive for *Arctium lappa* is arctigenin (AR), and its glucosylated form arctiin (Gao et al., 2018). In the Chinese Pharmacopoeia, arctiin is listed as a chemical marker and major active chemical ingredient in Fructus arctii (Chinese Pharmacopoeia, 2010). Regarding the pharmacological properties, arctigenin has antitumor and antidiabetic activities, while the derivatives isolappaol C, lappaol (C, D, F), and diarctigenin have anti-inflammatory activities. Biological tests revealed that arctigenin has A.A. and reduces both inflammation and level of lipids (Thaisa et al., 2020; Chen et al, 2020; Chena et al., 2020). Arctigenin (AR) and arctiin inhibit microorganisms and viruses; the mechanism of the anti-influenza effect of AR has been linked to the direct inhibitory effect on

viral replication (Hayashi et al., 2010; Yuan et al., 2008; Swarup et al., 2008). Inflammatory responses play an important role in the progression of many chronic diseases, so AR can serve as adjuvants in the treatment of these.

The main anti-inflammatory mechanism of AR is achieved by inhibiting the inducible synthesis of nitric oxide (iNOS), along with the modulation of proinflammatory cytokines (Tabas et al., 2013). The compound named arctigenin (AR), the most potent bioactive component found in *A. lappa* represents a promising therapeutic compound that can be used in the management of inflammation (Yao et al., 2011; Gao et al., 2018). Concerning the mechanism involved, studies performed with arctigenin on MH60 cell lines indicated that this compound stimulates the process of apoptosis (IC50 = 1.0 μM) as the most likely mechanism of action (Tousch et al., 2014; Ferracane et al., 2010). The mixture of arctigenin and polyphenols inhibit the development of the malign cells and decrease the apparition of metastases (Tousch et al., 2014); the compounds arctiin, caffeine, and chlorogenic acid all taken together have antimutagenic properties (Ferracane et al., 2010). Research performed *in vitro* with bioproducts obtained in CH_2Cl_2 from *A. lappa*, are cytotoxic for tumoral cells lines which have not received nutrients, at a concentration of 50μg brut extract/mL (Awale et al., 2006). The cell tumor cell lines type PANC-1 had a significantly higher resistance to nutrient deprivation and survived in these conditions for more than 48 hours. The researchers found that in the presence of arctigenin in the culture medium at a concentration of 0.01μ/mL tumor cells enter the process of apoptosis (Awale et al., 2006). Regarding the mechanism of action, preclinical studies performed in laboratory, animals with induced pancreatic cancer indicate that arctigenin (0.1 μg/ml) acts as an inhibitor of the Akt (Protein kinase B) phosphorylation process stimulated by the deprivation of glucose (Awale et al., 2006). Other studies confirmed the anti-tumor activity of *A. lappa* on tumor cell lines of the type: HepA; S180; MCF-7; BGC-823 (Agha et al., 2020). Matrix metallopeptidases (MMPs, zinc-dependent enzymes), play a central role in metastases (Lou et al., 2017). If the tumor cell lines type MDA-MB-231 (breast cancer) are exposed to AR the MMP-2 and MMP-9 activities are inhibited (Lou et al., 2017). Arctigenin enhances the processes of apoptosis in tumoral cell lines exposed to cisplatin, by acting as a sensitizer of cancer cells to cisplatin (Yao et al., 2011). Another study found that blood pressure was reduced in arctigenin-treated hypertensive rats (Liu et al., 2015). Ravini and collab. reported that arctigenin represents a cytotoxic compound for tumor lines, inducing their necrosis (Ayoddhia et al., 2019). The hydromethanolic extract made from *A. lappa* fruits exhibits a strong antitumor effect on MH60 cell lines, due to the presence of AR. Other researchers found that arctiin has a strong cytotoxic effect on the following tumor cell lines: HepG2; A549; K-OV-3; SK-MEL-2; XF498; HCT15 (Ferracane et al., 2010). Clinical trials were performed in Japan regarding the toxicity and safety profile of GBS-01 (an oral drug derived from *A. lappa* fruit which contains a high level of AR). In this study, the patients with pancreatic cancer with resistance at gemcitabine were received AR in doses ranging between (3÷12) g/day. In the first 4 weeks, the established degree of toxicity was 4, with non-hematological toxicity, Following the studies performed, it was observed a slight increase of the levels of the gamma-glutamyl transferase, serum glucose level, and total bilirubin (Strimpakos et al., 2013). A greater number of studies over time have focused on polysaccharides extracted from *A. lappa*, especially fructans, which have been used as a source of inulin (Watanabe et al., 2020). Recently, natural lignans from *A. lappa* have been found to have promising antitumor potential. They can induce apoptosis in cancer cells, suppressing tumor growth by decreasing tumor tolerance to glucose, leading to starvation (Watanabe et al., 2020). A recent study found that a bioproduct derived from *A. lappa*, obtained by extraction in an aqueous solution of 70% EtOH, exerted inhibitory effects on atherogenic diet-induced thickening of the vascular wall in the aorta (Song et al., 2018).

CONCLUSIONS

Data from the literature have confirmed that *Arctium lappa* contains a large number of bioactive components, with definite beneficial biological effects. In this context, arctigenin

(AR), the most potent bioactive component found in *A. lappa*, is a promising therapeutic compound that can be used in the management of both acute inflammation and chronic inflammation. Therefore, studies have shown a wide range of possible clinical uses of this plant due to its anti-inflammatory, antitumor, antiviral and antimicrobial effects.

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