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Edited by
Alina Maria Holban
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Therapeutic Foods

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CHAPTER 10

Perspective Therapeutic Effects of Immunomodulating Acidic Herbal Heteropolysaccharides and Their Complexes in Functional and Dietary Nutrition

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Abbreviations

AG	Arabinogalactan
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
BPH	Prostatic hyperplasia
BW	Body weight
DTH	Delayed-type hypersensitivity
EAE	Autoimmune encephalomyelitis
5-FU	5-Fluorouracil
GID	Gastrointestinal disorders
GIT	Gastrointestinal tract
GSH-Px	Glutathione peroxidase
HG	Homogalacturonan
IBD	Inflammatory bowel disease
LCC	Lignin-carbohydrate complex
LMWC	Lower molecular weight compounds
MCP	Modified citrus pectin
MDA	Malondialdehyde
PBMC	Peripheral blood mononuclear cells
PI	Prebiotic index
RG	Rhamnogalacturonan
SCFA	Short-chain fatty acids
SOD	Superoxide dismutase
SRBC	Antigen sheep red blood cells
TBARS	Thiobarbituric acid reactive substances

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1 Introduction

1.1 Trends in Herbal Medicine and Nutrition

Humans and animals have approached instinctively their health maintenance that relies on medicinal plants since time immemorial. Nowadays people can partly explain the physiological effects of a given herb and use its therapeutic potential by isolating its active constituents, proving their mode of action and developing a drug or a nutraceutical product. There are about 300,000 species of higher plants in the world, and only between 6% and 15% of them are investigated for bioactive compounds. Medicinal herbs that are exploited in phytotherapy are derived from more than 53,000 plant species (Pan et al., 2014). For example, Bulgaria has about 770 species of medicinal plants, and 200 are currently in use as more than 250 herbal drugs are produced from them and used in the prophylaxis, medicine, cosmetics, and the food industry (Evstatieva et al., 2007). According to the World Health Organization (WHO), 75% of the human population uses herbs for health care. Eleven percent of those evaluated by WHO as extremely important medicines are produced only by plants. For instance, the 2015 Nobel Prize in Physiology or Medicine was awarded half to the discovery of a plant drug artemisinin that saved millions of lives from malaria. About 60% of the antitumor drugs in clinical trial phase are of natural origin (Demain and Vaishnav, 2011). More than 70% of German physicians use in their practice plant-derived drugs and phytotherapeutics are even covered by the national health insurance in Japan (Azaizeh et al., 2010). Research and development studies on immunomodulating and antioxidant therapeutic effects of medicinal herbs have always been intriguing scientists, medical practitioners, producers, and entrepreneurs (Cravotto et al., 2010; Kratchanova et al., 2010a; Labadie et al., 1989; Liu et al., 2015b; Radoucheva et al., 1995; Ramberg et al., 2010; Wagner et al., 1999). Medicinal plants are the main and most perspective raw material for the growing nutraceutical industry (Kathirvel et al., 2015). Interestingly, green tea from *Camellia sinensis* is the most widely consumed liquid after water in the world (Nie and Xie, 2011). According to Transparency Market Research, the global nutraceutical market was valued at US $165.62 billion in 2014 and is expected to reach US $278.96 billion by 2021.

1.2 Importance of Bioactive Acidic Heteropolysaccharides

In a classical manner, most of the therapeutic effects of medicinal plants are related to their lower molecular weight compounds (LMWC), which are accepted as the most active constituents. For more than 30 years of scientific research in Chinese medicine, and especially in Japanese Kampo medicine, traditional European, African, and Indian Ayurvedic medicines has been proving that acidic herbal polysaccharides are also main contributors to biological activity of almost every herb (Liu et al., 2015c; Paulsen and Barsett, 2005; Popov and Ovodov, 2013; Srivastava and Kulshreshtha, 1989; Thakur et al., 2012; Wangensteen et al., 2015; Yamada, 1994, 1996; Yamada and Kiyohara, 2007). At the same

time, old medicinal books recommend for gastrointestinal disorders (GID) herbs like *Althea officinalis*, *Tanacetum vulgare*, and *Rheum palmatum*, which are currently well-known rich sources of acidic polysaccharides (Nedelcheva, 2012). On a cellular level in plants, acidic heteropolysaccharides and their conjugates are physiologically active dynamic structural blocks of the cell wall and intercellular matrix. They play a crucial role in cell growth, proliferation, and defense (Knox, 2002). All human cells express carbohydrates on their surfaces in the form of glycoproteins, glycolipids, and oligosaccharides that are important for immunological response and numerous primary biological functions (Nangia-Makker et al., 2002). From a physiological point of view, the human organism has created an adequate adaptation to acidic plant polysaccharides because they are present in the normal diet through fruits, vegetables, and herbs. Although herbal acidic polysaccharides do not have any nutritional value for humans, they are natural functional ingredients in our traditional foods and drinks, and remedies used in the folk medicine. These polysaccharides are famous for the lack of or low cytotoxicity and detrimental side effects, and are degraded in the cecum, ascending and transverse colon by residential microflora to bioactive short-chain fatty acids (SCFA) (Endress, 2011).

The family of herbal acidic heteropolysaccharides includes mainly two groups: pectins and acidic hemicelluloses. Both groups are referred to as mucilage and gums by pharmacists or to dietary fibers by nutriologists. Because of the cell wall complexity, negative charge, and extraction conditions these acidic polymers are isolated quite often in the form of covalently or physicochemically bound conjugates with glucans, proteins, lignins, polyphenols, and minerals (Kiyohara et al., 2000; Nie and Xie, 2011; Pawlaczyk et al., 2009). Special attention should be given to pectic polysaccharides, whose importance for maintenance of human health is determined by their beneficial effect on digestive, urinary, cardiovascular, endocrine, nervous, and respiratory systems (Chen et al., 2011; Endress, 2011; Šutovská et al., 2013; Wicker et al., 2014). Braconnot (1825) proposed the application of pectins in medicine because of its jellifying properties. The therapeutic effect of pectin in the famous apple diet of Heisler-Moro for the relief of GID was explained in 1931 by Malyoth (Kertesz, 1951). The first evidence-based study that showed clearly the cholesterol-lowering action of pectin was published in 1961 (Keys et al., 1961). Generally, acidic polymers express in vitro and in vivo anticomplementary, lymphocyte, and phagocyte modulating, antitumor, antiinflammatory, anticoagulant, prebiotic activities, and so on (Bijak et al. 2013; Ferreira et al., 2015; Paulsen, 2001; Yamada and Kiyohara, 2007). It is proved that after oral administration immunoactive herbal polysaccharides stimulate the intestinal immune system through interaction with lymphocytes in Peyer's patches, which further have a complex impact on the systemic immune system (Yamada and Kiyohara, 2007). Moreover, pectins form hydrocolloids in the gastrointestinal tract (GIT) that absorb steroids, lipids, hydrolytic enzymes, and other metabolites, which are further excreted from the body (Groudeva et al., 1997; Stantschev et al., 1979). Classical examples of immunomodulating

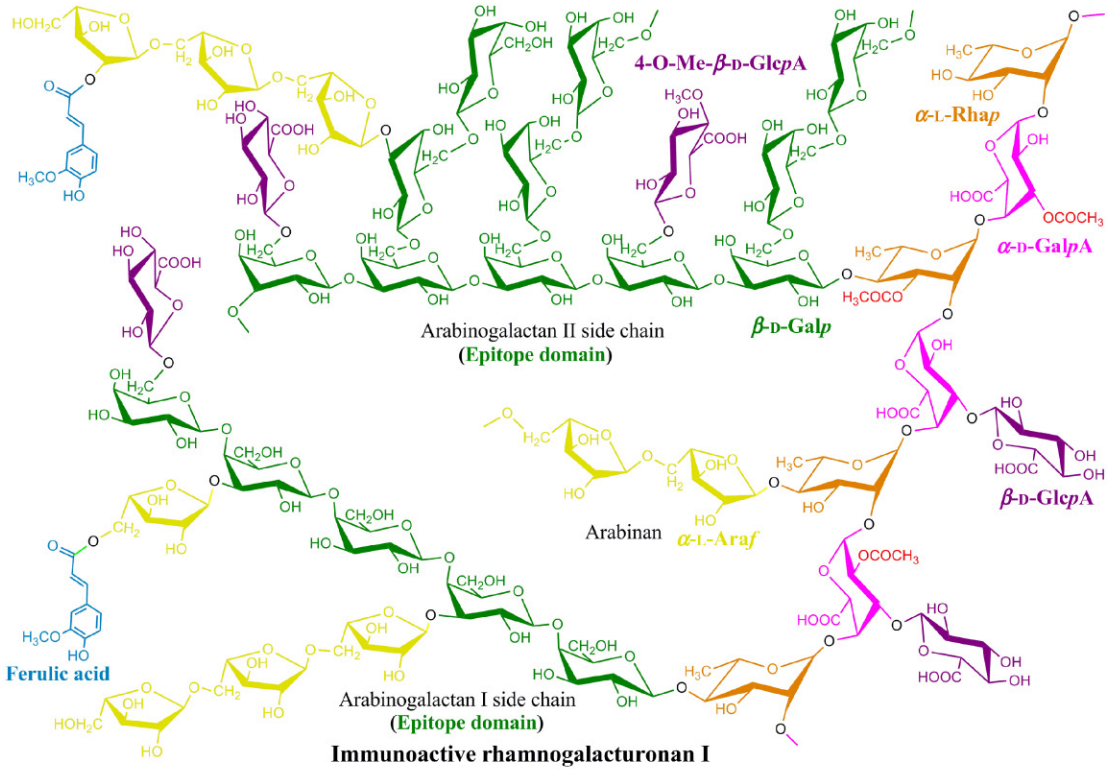
acidic polysaccharides are pectic polysaccharides isolated from *Angelica acutiloba*, *Urtica dioica*, *Bupleurum falcatum*, *Viscum album*, *Biophytum petersianum*, *Echinacea purpurea*, *Plantago major*, leek, citrus fruits, and glucuronoarabinoxylans from *E. purpurea* and *P. major* (Barsett et al., 2012; Georgiev et al., 2012; Inngjerdingen et al., 2008; Kratchanova et al., 2010b; Matsumoto et al., 2008; Mueller and Anderer, 1990; Ognyanov et al., 2014; Proksch and Wagner, 1987; Samuelsen et al., 1996, 1999; Wagner et al., 1989, 1994; Yamada et al., 1985, 1989). As a result of these continuous and comprehensive studies the immunomodulating therapeutic effects of crude herbal drugs were partly explained, leading to further development of numerous food supplements for treatment of different kinds of immunodeficiency from influenza to tumor diseases, as well as for regular detoxification and maintenance of human body. An extensive collaborative study on Malian medicinal plants focused on traditional wound healing water extracts revealed important role of immunomodulating pectic polysaccharides on the activity of traditional formulations (Wangensteen et al., 2015). Good commercial and clinically tested examples are crude mistletoe extracts, nettle root extracts, Kampo medicine formulations Juzen-taiho-to, Hochuekkito, *Astragalus* polysaccharide extract, and enzyme modified citrus pectin PectaSol-C (He et al., 2012; Kiyohara et al., 2004; Morris et al., 2013; Sekiya et al., 2013). Interestingly, it was found that the quality of life of cancer patients supplemented with apple pectin in parallel with chemo- and radiotherapy was improved (Botushanova et al., 1999; Tomova et al., 1999; Yaneva et al., 2002). In addition, functional drinks on the basis of chokeberry juice enriched with pectin and chokeberry juice with rosehip extract were developed in our laboratory and currently produced by Vitanea Ltd. These functional drinks express cardioprotective and antiatherogenic effects (Daskalova et al., 2015). Generally, acidic heteroglycans expand their role as technological food hydrocolloids to biofunctional food additives.

2 Chemical Diversity of Herbal Acidic Heteropolysaccharides and Relation to Immunogenicity

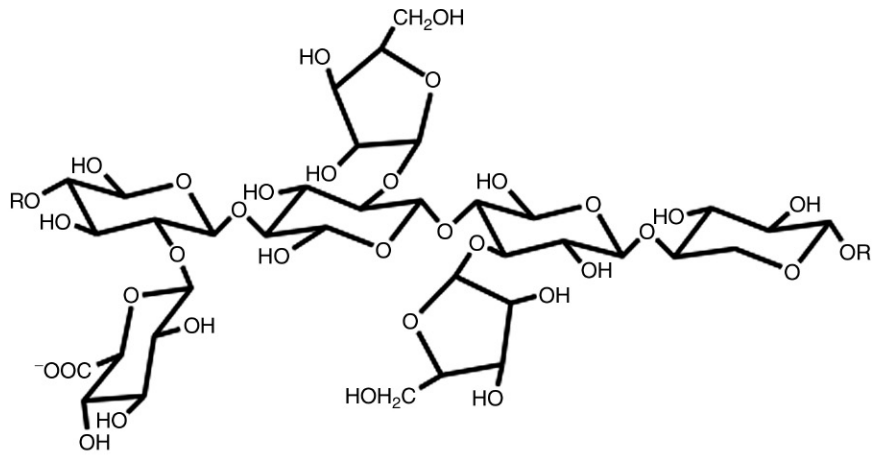
As already stated, medicinal plants contain different in structural complexity, charge and function homo- and heteropolysaccharides, such as cellulose, other glucans (starches, β-glucans), inulin, pectic polysaccharides, hemicelluloses (xylans, arabinoxylans, glucuronoarabinoxylans, xyloglucans, glucomannans), and their complexes with other compounds (Ebringerová et al., 2005; Liu et al., 2015c; Nie and Xie, 2011; Paulsen and Barsett, 2005; Thakur et al., 2012).

Pectic polysaccharides are the most complex heteroglycans on Earth. Although they were discovered 226 years ago (Vauquelin, 1790), polymer fine structure is still an open issue. Generally, pectic polysaccharides are composed of about nine different monosaccharides (Ara, Gal, Rha, Xyl, Fuc, Glc, 2-Me-Fuc, 2-Me-Xyl, and Api), three uronic acids (GalA,

GlcA, and 4-O-Me-GlcA), two ulosonic acids (Kdo and Dha) and one 3-carboxy-5-deoxyxylose (Ace) in certain anomeric, enantiomeric, conformational and substituted forms. On a structural point of view, pectins contain three main building blocks: unbranched homogalacturonan, ramified rhamnogalacturonan I, and II. Some galacturonans branched with Xyl, Api, etc. also occur (Albersheim et al., 1996; Schols and Voragen, 2002; Voragen et al., 1995). The most abundant is homogalacturonan (HG), followed by rhamnogalacturonan (RG) I. Linear HG is composed of α-(1→4)-linked poly-D-Gal*p*A, which carboxyl groups are methyl-esterified to different extent and some hydroxyl groups at C-2 and/or C-3 can be acetylated (Yapo, 2011a). Furthermore, it is covalently linked by an α-(1→2)-glycosidic bond with the linear repeating fragment [→2)-α-L-Rha*p*-(1→4)-α-D-Gal*p*A-(1→]$_n$ that represents RG I backbone (Yapo, 2011a). The latter carries side chains of α-(1→5)-L-arabinans, β-(1→4)-D-galactans, arabinogalactan types I, and II at a specific branching point, -C-4 hydroxyl group of L-Rha residues (McNeil et al., 1980; Voragen et al., 2009). It is found for herbal pectins that GalA units are partly or highly substituted at O-3 with β-D-Glc*p*A units (Georgiev et al., 2017b). Similarly, Rha units are rarely acetylated at O-3 (Sengkhamparn et al., 2009; Georgiev et al., 2017b). The presence of methyl and acetyl ester groups in the neutral side chains of RG I backbone could not be excluded. Togola et al. (2008) reported a pectic polysaccharide with 40% mono-*O*-methylated monosaccharides in RG I side chains. Arabinogalactan (AG) I is an arabino-4-galactan, composed of a linear β-D-(1→4)-linked galactan (Gal*p*), which is branched at O-3 with mainly α-L-(1→5)-linked Ara*f* residues. AG II is an arabino-3,6-galactan composed of β-D-(1→3)-linked galactan backbone with side chains at O-6 of β-(1→6)-linked galactans. 1,3- and 1,6-galactans are substituted to different extent mainly with α-L-(1→3)-linked Ara*f* residues (Schols and Voragen, 2002). Glycosidic coupling of L-Ara in AG structures is highly diverse (1,2; 1,3; 1,5). Usually AG side chains are terminated with β-D-Glc*p*A, 4-O-Me-D-Glc*p*A, α-L-Fuc*p*, and other sugars (Matsumoto et al., 2008; Yapo, 2011a). Additionally, α-L-Ara*f* and β-D-Gal*p* residues could be esterified with ferulic acid at O-2, O-3, O-5, and O-6 respectively (Ralet et al., 2005). The complex chemical structures of RG I side chains or direct AG I and II structures are extremely important for the expression of immunomodulating activity (Yamada and Kiyohara, 2007). It is well known that RG I in herbal pectic polysaccharides is strongly ramified and it often contains AG II side chains. AG II structural motifs are of great importance for recognition from immunocompetent cells (Ferreira et al., 2015). This could be clearly seen from the works of Yamada and Kiyohara, and Paulsen (Paulsen, 2001; Yamada and Kiyohara, 2007). Fig. 10.1A presents a model structure of immunoactive RG I with AG I and II side chains. Furthermore, it is very common that the distribution ratio between HG and RG I in herbal pectic polysaccharides is lower than in fruit pectin (Ognyanov et al., 2016; Wangensteen et al., 2015). In fact, herbal pectins often have a lower molecular weight than those from fruits. Sometimes authors use the term acidic AG for native herbal pectic fractions (almost) lacking HG (Wang et al., 2005). Generally, these types of heteroglycans express very potent



(A)



(B)

Figure 10.1: (A) A model structure of immunoactive rhamnogalacturonan I with arabinogalactan I and II structural motifs. (B) The basic structure of glucuronoarabinoxylan (Caffall and Mohnen, 2009).

activity. The literature data on the importance of HG for expression of immunomodulating activity are still contradictory (Yamada and Kiyohara, 2007). Maybe the HG region could serve as a dynamic arm like in the plant cell wall for better interaction of AGs with biological receptors.

The third main structural pectic fragment is RG type II that is covalently linked for HG by α-(4→1)- and/or α-(1→1)-glycosidic bonds at the nonreducing end (Bar-Peled et al., 2012; Darvill et al., 1978). It has an extraordinary and conservative complex composition and structure. Generally, RG II is composed of a partly methyl-esterified galacturonan backbone, carrying four oligosaccharide side chains rich in rare sugars (2-Me-Fuc, 2-Me-Xyl, Api, Kdo, and Dha). RG II exists in nature as a dimer formed by borate-diester linkages (O'Neill et al., 2004). RG II has been found to be relatively abundant in red wine and other fermented beverages (Buffetto et al., 2014). This dimeric form is physiologically active for both plants and humans (Li et al., 2014b). At the same time dimeric RG II is absorbent for heavy metals and the number of publications for its immunomodulating properties is increasing (Yapo, 2011b). Shin et al. (1997) showed that RG II from the leaves of *Panax ginseng* could be macrophage Fc receptor expression-enhancing polysaccharide. Later, they reported for the first time that dissociation of the RG II dimer led to a decrease in the expressed immunomodulating activity against macrophages (Shin et al., 1998).

Elucidation of structure-immunomodulating activity relationship of pectic polysaccharides is realized by using pectinolytic enzymes (Georgiev et al., 2013; Sakurai et al., 1998; Voragen et al., 2009; Yamada and Kiyohara, 2007). Enzyme modification is combined with SEC and further GC-MS glycosidic linkage composition analysis and LC-EIS-IT-MS/MALDI-TOF-MS or 2D NMR for sugar sequencing (Li et al., 2014b; Ognyanov et al., 2016; Sakurai et al., 1998; Sengkhamparn et al., 2009; Zou et al., 2014a).

Another bioactive herbal acidic heteropolysaccharides are hemicellulosic glucuronoarabinoxylans. They are acidic heteroxylans composed of linear β-(1→4)-linked D-Xyl*p* units substituted with α-(1→3)-linked L-Ara*f* residues and α-(1→2)-linked D-(4-O-Me)Glc*p*A residues (Fig. 10.1B). Xyl*p* residues could be methylated and/or acetylated and Ara*f* residues could be feruloylated or coumarylated (Ebringerová et al., 2005).

During cell growth and extraction or decoction preparations polysaccharides can form immunoactive complexes with noncarbohydrate compounds that could influence immunomodulating properties of the former polymer. For example, acidic heteropolysaccharides can interact with phenolic acids and polyphenols, especially flavonoids: procyanidins, anthocyanins, and catechins. These interactions are based on the formation of weak noncovalent bonds (hydrogen bonds and hydrophobic interactions), covalent linkages, and adsorption. Hydrogen bonds are formed between –OH groups of polyphenols and –O- from the glycosidic linkages (Jakobek, 2015). Covalent ester bonds

are formed predominantly with phenolic acids (Ralet et al., 2005). Hydrophobic pocket or cavity formation in polysaccharide macromolecule is important for hydrophobic interactions with procyanidins (Watrelot et al., 2014). Interestingly, we separated from lavender pectins after an exhaustive ethanol treatment and SEC the ethanol-soluble catechin, epicatechin, quercetin, and other flavonoids (Georgiev et al., 2017c). Acidic polysaccharides can form glycoconjugates with lignins, as well (Sakagami et al., 2010). The chemistry of this interaction is still poorly understood but information on the composition of both separate parts is available. Generally, the carbohydrate part is a pectic polysaccharide or neutral heteroglycan. Lignin part is represented by vanillin, guaiacol, syringaldehyde, *p*-hydroxybenzaldehyde, *p*-hydroxyacetophenone, cinnamic acid derivatives, and so on (Bijak et al., 2013; Kiyohara et al., 2000). Varying the ratio of polysaccharide to phenylpropenoids produces heterogeneity in the acidity, water-solubility, ethanol-insolubility, and molecular weight (Sakagami et al., 2010). Sakagami et al. (1987) reported that antitumor activity of lignin-carbohydrate complex (LCC) increased with the acidity. They also concluded that polymerized structure of phenylpropenoids in LCC is required for the induction of antiviral activity, and that the higher-order structure of phenylpropenoid polymers and polysaccharides is required for immunopotentiation. Kiyohara et al. (2000) demonstrated that ex vivo intestinal immune system modulating activity of LCC was significantly reduced by $NaIO_4$ or $NaClO_2$ treatments, concluding that the whole glycoconjugate was important for the activity. Additionally, herbal polysaccharides were found to be mostly conjugated with proteins where carbohydrate moiety is covalently attached to a polypeptide backbone usually via *N*- or O-linkages (Nie and Xie, 2011). For example, AG II is often linked to a protein moiety through Hyp or Ser residues (Tan et al., 2010). Zhang et al. (2011) reported that after digestion of protein moiety in polysaccharide-protein complex it lost activation ability on T-lymphocytes.

Fig. 10.2 presents our own recent results on the distribution of acidic (pectic) polysaccharides in 4 different organs of 34 widely used European medicinal plants. Acidic polysaccharides in herbs were estimated on the basis of anhydrouronic acid content (AUAC), corresponding to the total uronic acids and GalA content in water-extracted, insoluble in 70% ethanol and dialyzed macromolecular complexes. All investigated plants contain pectic polysaccharides, as their distribution is higher in fruits and flowers and lower in leaves and roots. The higher difference between AUAC and GalA amount in the linden sample was explained by the presence of a reasonable amount of GlcA in it. Later, it was proved by GC-MS methylation analysis and 2D NMR study that silver linden flowers contain a novel glucurono-rhamnogalacturonan I that is acetylated at O-3 of Rha units (Georgiev et al., 2017b). On the basis of sugar composition, determined by the corresponding alditol acetates, the crude herbal polysaccharides contain typical for pectins (Rha, Ara, Gal, Xyl, Fuc) and for other water-extractable herbal polysaccharides (Glc, Man) monosaccharides. Additionally, the predominant molecular weight is $\leq$100 kDa, as determined by HPSEC-RID analyses (Georgiev et al., unpublished results).

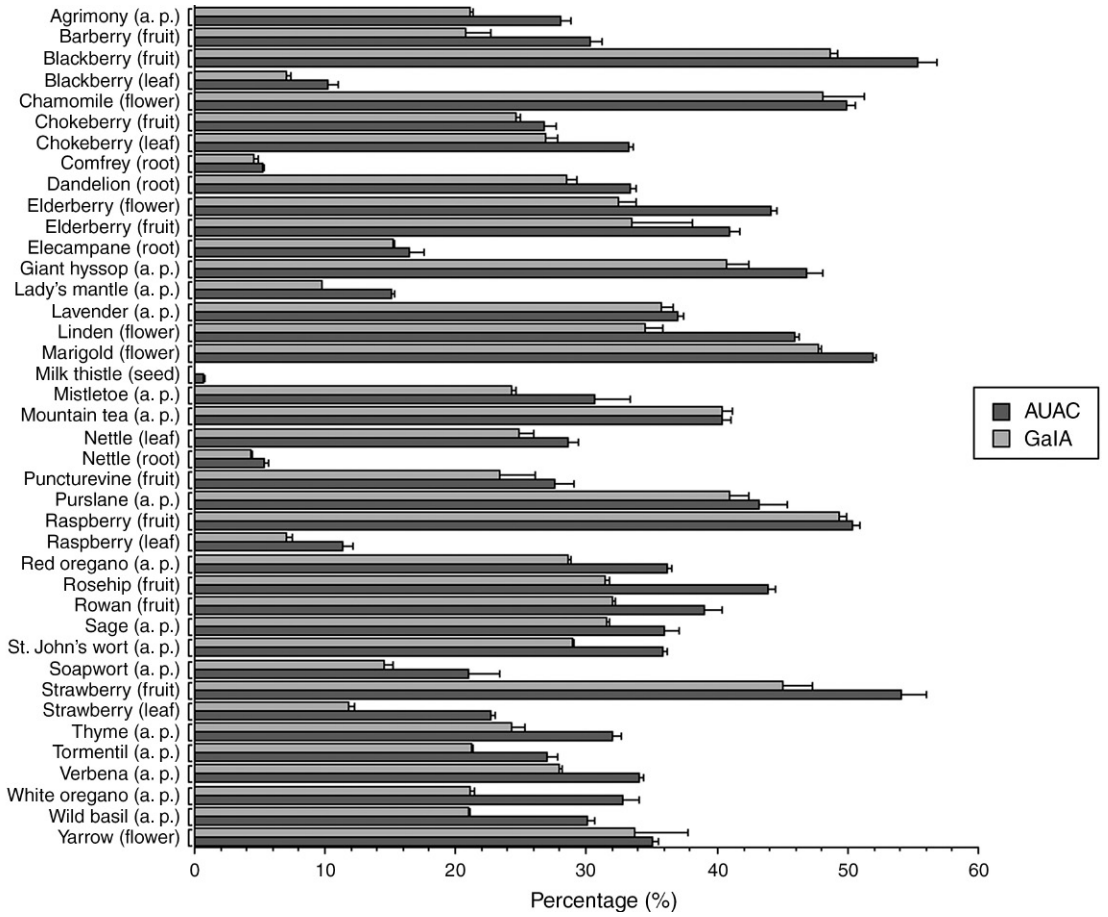


Figure 10.2: Anhydrouronic Acid Content (AUAC) and Galacturonic Acid (GalA) Content of Water-Extracted Crude Polysaccharides From Different European Medicinal Plants.

3 Perspective Therapeutic Effects of Acidic Heteropolysaccharides

3.1 Modulation of Innate and Adaptive Immunity

Originally, the health-promoting properties of medicinal plants and their extracts have been documented for centuries (Azaizeh et al., 2010; Nedelcheva, 2012; Pan et al., 2014). Later, some of these properties were specified as immunomodulating activities and now polysaccharide research revealed that carbohydrates are important for the observed effects (Wagner et al., 1999). More and more polysaccharides isolated from medicinal plants are reported for their potent immunomodulating activities (Ferreira et al., 2015). Immunomodulating activity of a given compound is explained by its pharmacological effects on humoral and cellular immunological factors. Regulatory interactions between these factors in the course of immune response from the immunomodulating compound

determine its immunostimulating or immunosuppressing effects (Labadie, 1993). Acidic heteropolysaccharides could mediate activity of certain immunological factors. Important factors from innate immunity are complement system, NK-cells, phagocytes (macrophages, neutrophils, dendritic cells), and their metabolites (monokines) and mediator cells (mast cells, basophils, eosinophils, and thrombocytes), producing proinflammatory molecules. Prominent immunomodulating cells from adaptive immunity are T- and B-lymphocytes, and their metabolites (immunoglobulins and interleukins) (Khan, 2008). The actual effects may be specific or nonspecific. For instance, acidic polysaccharides can stimulate phagocytes through interaction with some surface molecules (receptors) and initiate phagocytosis, production of NO, ROS, cytokines, etc. (Lojek et al., 2014). Matsumoto et al. (1993) and Matsumoto and Yamada (1995) demonstrated that pectic fraction bupleuran 2IIb from *B. falcatum* could enhance immune-complex binding to peritoneal macrophages through Fc receptor by a mechanism dependent on an increase in intracellular Ca^{2+}, followed by activation of the calmodulin, but not by a PKC or PKA pathway. Rhamnogalacturonyl arabinogalactans react with serum IgG and form a complex that initiates complement activation through the classical pathway (Kiyohara et al., 2006; Yamada et al., 1991). Furthermore, ramified pectic regions rich in AG II are recognized by certain receptors in lipid rafts of B-cells, which induce activation and proliferation of B-cells (IgM and IL-6 production) (Matsumoto et al., 2008; Sakurai et al., 1999). Therefore, polysaccharides could modulate innate and adaptive immune systems, which open their great potency for the development of new therapeutics and agents beneficial to human health. Fig. 10.3 shows a schematic overview of perspective therapeutic effects from immune system modulation by supplementation with herbal polysaccharides, which are further presented in the next sections.

3.1.1 In vitro complement system modulation

Some of the most studied immunomodulating effects of pectic polysaccharides are those on the complement system. The complement system is a vital instrument for the innate immunity and particularly general antimicrobial protection mechanism for humans (Austarheim et al., 2012a; Ehrnthaller et al., 2011). The complex pectic structure is recognized by the complement, similar to recognition of microbial cell wall polysaccharides, thus acting as an antigen-mimetic. Pectins activate complement system through the classical and alternative pathways (Michaelsen et al., 2000; Yamada et al., 1991). Complement activation is a complex immunological process in which different immunocompetent cells like macrophages, NK, T-, and B-cells are involved and activated. Therefore, pectins stimulate innate immune response and host defense through the complement. Generally, polysaccharides, expressing such effect, are termed anticomplementary or complement-fixing compounds (Wangensteen et al., 2015; Yamada and Kiyohara, 2007). Their effect is evaluated on inhibition of total complement hemolysis of targeted animal erythrocytes in vitro, as this evaluation system is not enough for differentiation between complement activation and inhibition. Interestingly, the classical view of complement as a major system of host defense that copes with infections

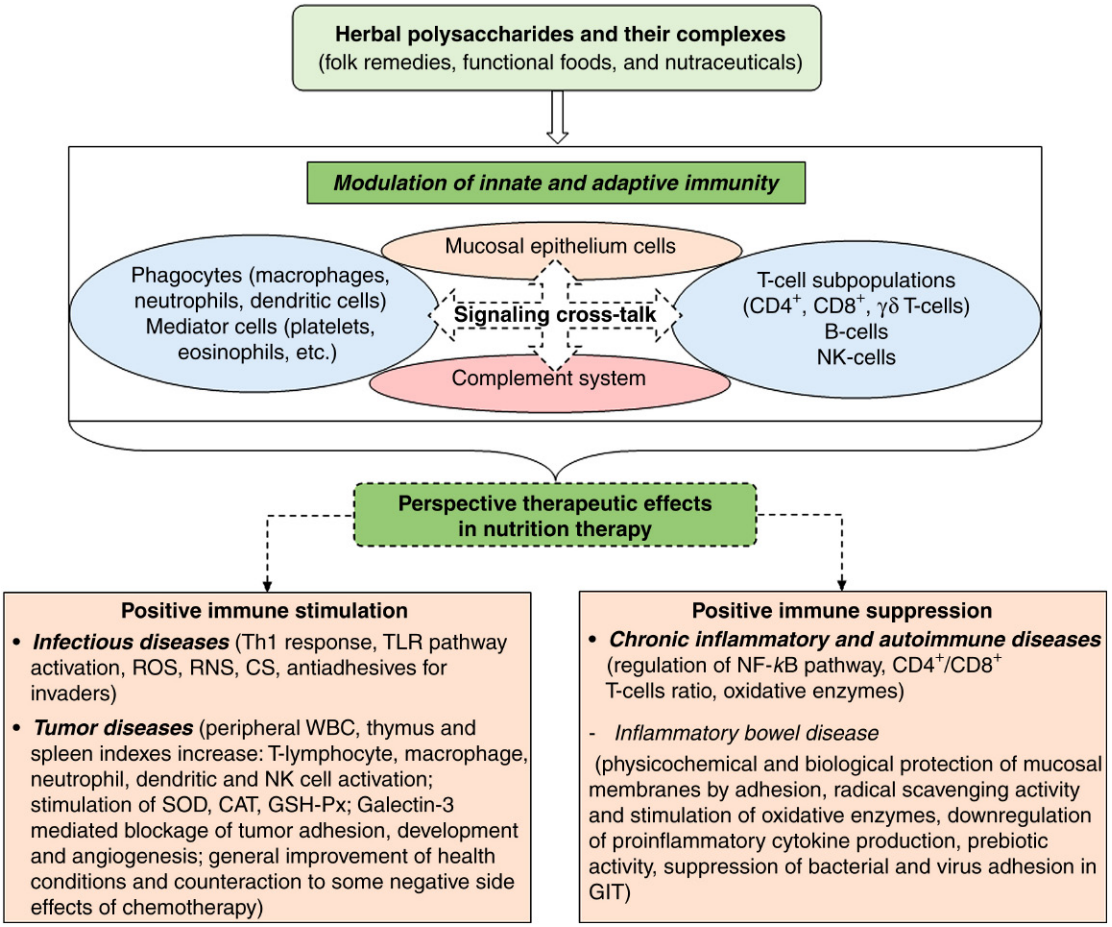


Figure 10.3: A Schematic Overview of Perspective Therapeutic Effects From Supplementation With Immunomodulating Herbal Acidic Heteropolysaccharides.

CS, Complement system; *GIT*, gastrointestinal tract; *GSH-Px*, glutathione peroxidase; *SOD*, superoxide dismutase; *WBC*, white blood cells.

is changing to that of a multifunctional player in tissue homeostasis, degeneration, and regeneration (Phieler et al., 2013). Complement modulation effect of acidic polysaccharides was extensively examined and reviewed by the groups of Yamada and Paulsen (Paulsen, 2001; Paulsen and Barsett, 2005; Yamada and Kiyohara, 2007) and some recently obtained literature results on this topic are reviewed here. For example, Zou et al. (2014a) obtained two pectic polysaccharide fractions 50WCP-II-I and 100WCP-II-I from the roots of *Codonopsis pilosula* with potent in vitro human complement fixation activity and $IC_{50} \approx 32.0$ μg/mL. 50WCP-II-I fraction presented long partly methyl-esterified HG region, interrupted by one short RG I with AG I and II side chains. Fraction 100WCP-II-I had two main ramified regions, one was unusual galacturonan region with AG-I side chain on position 2 of GalA, and the other

one was RG-I region with AG-II side chain. The authors concluded that responsibility for complement fixation activity in two fractions were mainly ramified regions on the basis of *endo*-polygalacturonase release of nonbranched galacturonans and reevaluation of biological activity of the corresponding subfractions. After enzyme treatment 50WCP-II-I-a subfraction expressed the highest activity with IC_{50} = 6.5 μg/mL, followed by 100WCP-II-I-a fraction with IC_{50} = 12.2 μg/mL. Zou et al. (2014b) also reported isolation of immunomodulating pectins from the bark of traditionally used in Malian medicine *Parkia biglobosa* that expressed potent complement fixation activity and stimulated macrophages to release NO. Zhang et al. (2014) isolated pectic polysaccharides from the leaves of the African plant *Lessertia frutescens* called "cancerbush" that possessed potent in vitro complement-fixating activity. These findings help for the elucidation of therapeutic effects of this traditionally used plant for treatment of cancer related illness. Košťálová et al. (2014) isolated two hemicellulosic neutral and acidic alkali-soluble fractions from the leaves of *Fallopia sachalinensis* that exhibited significant immunostimulating activity in the complement-fixation test. Similarly, Ho et al. (2015) showed that elderberries contain pectic polysaccharides with potent dose-dependent in vitro complement fixating activity and macrophage stimulating activity, as responsible for the immunomodulation were ramified regions. Interestingly, pectins from 100°C extract were more active. In another study on pectins from elderflowers they obtained higher immunomodulating activity and observed enhancement of bioactivity after removal of ester groups by NaOH. The authors concluded that their results could partly explain therapeutic effects of elderflowers in colds and influenza (Ho et al., 2016).

3.1.2 Intestinal immune system modulation

When orally administered, polysaccharides are transported through the stomach to the small intestine and colon. Immunostimulating polysaccharides modulate the intestinal immune system, which has been known to direct not only defensive but also regulatory functions of both mucosal and systemic immune systems through lymphocyte migration depending on homing receptor pattern (Jung et al., 2010). It was demonstrated by animal models that herbal polysaccharides stimulate the intestinal immune system through interaction with lymphocytes in Peyer's patches, which further have a complex impact on the systemic immune system (Yamada et al., 2009). For instance, Yu et al. (1998) isolated from rhizomes of *Atractylodes lancea* three pectic polysaccharide fractions with potent ex vivo intestinal immunomodulating activity against murine Peyer's patch cells. The most active ALR-5lla-1-1 fraction was rich in AG II and digestion with *exo*-α-L-arabinofuranosidase and *exo*-β-D-(1→3)-galactanase significantly decreased its activity. Kiyohara et al. (2002) demonstrated that oral administration of the isolated pectic polysaccharide fraction F-5 from the Japanese herbal prescription, Juzen-Taiho-To (150 mg/kg/day) to mice enhanced function of Peyer's patch cells. The increased activity after *endo*-polygalacturonase treatment of F-5 and further structural studies proved that the most potent intestinal immune system modulating activity was expressed by AG II-rich fractions. Kiyohara et al. (2010) obtained

14 pectic fractions from *A. mongholics* and 13 of them showed high dose-dependent ex vivo immunomodulating activity toward cells from Peyer's patches at 10, 50, and 100 μg/mL. The activity was sharply reduced by enzyme degradation of β-[1→3(6)]-D-galactans. Similarly, Grønhaug et al. (2011) showed by specific β-D-galactanases that β-(1→4)- and β-[1→3(6)]-D-galactans were important for the immunomodulating activity of pectin from *B. petersianum* against intestinal Peyer's patch cells and macrophages ex vivo. Suh et al. (2013) reported a pectic polysaccharide CUI-3IIb-3-2 from peels of *Citrus unshiu*, expressing a powerful immunomodulating activity on T- and B-cells from Peyer's patches. The polysaccharide was losing its activity after *endo*-polygalacturonase modification. It also increased proliferation of bone marrow cells in C3H/He mice at 1 g/body weight (BW) and GM-CSF/IL-6 production from T- and B-cells from Peyer's patches. Slavov et al. (2013) found that water-soluble pectin from waste petals of Bulgarian rose expressed ex vivo intestinal immune system modulating activity through Peyer's patch. Later, in our study on the search of immunoactive compounds from lavender, we found that lavender pectic polysaccharides contribute to the immune-enhancing properties of lavender. Pectic fraction chPS-L1 rich in AG II was free of LPS and expressed anticomplementary activity through the classical and alternative pathways with IC_{50}values of 94 and 38.9 μg/mL, respectively. At a concentration of 100 μg/mL, it also activated murine Peyer's patch immunocompetent cells, NO production from murine macrophages and spontaneous ROS production from whole blood and isolated human neutrophils. chPS-L1 showed ≥20% in vitro inhibition of A549 human lung adenocarcinoma, LS180 colon adenocarcinoma, and Jurkat T cell leukemia at 50 μg/mL after 48 h incubation (Georgiev et al., 2017c). Production of lavender oil and lavender-based products for maintenance of human health is increasing worldwide and lavender-derived nutraceuticals could be effective for improvement of human health. For illustration, Fig. 10.4A presents active carbohydrate chain units against Peyer's patch immunomodulation.

After immunomodulating polysaccharides are thought to incorporate into Peyer's patches through M-cells by pinocytic manner and/or as secretory IgA-polysaccharide complexes through polymeric immunoglobulin receptors, they are postulated to interact with immunocompetent cells, such as dendritic cells, macrophages, stromal cells and B cells. Polysaccharides are fairly well absorbed by intestinal epithelial cells, but their communication with the epithelium could also lead to immunomodulation of underlying dendritic cells because epithelial cells have been known to possess functions of immunocompetent cells. By these dual modes of action of active polysaccharides, functionally modulated T- and B-lymphocytes from Peyer's patches and dendritic cells from lamina propria interact in mesenteric lymph nodes. These lymphocytes are proposed to result in modulation of several mucosal and systemic immune systems with their by-stander effect in lymphoid and nonlymphoid organs through lymphocytes homing phenomenon (Jung et al., 2010; Tang et al., 2015; Yamada and Kiyohara, 2007). This mechanism is shown on Fig. 10.4B. The proposed mode of action of herbal polysaccharides on intestinal immune system is logically

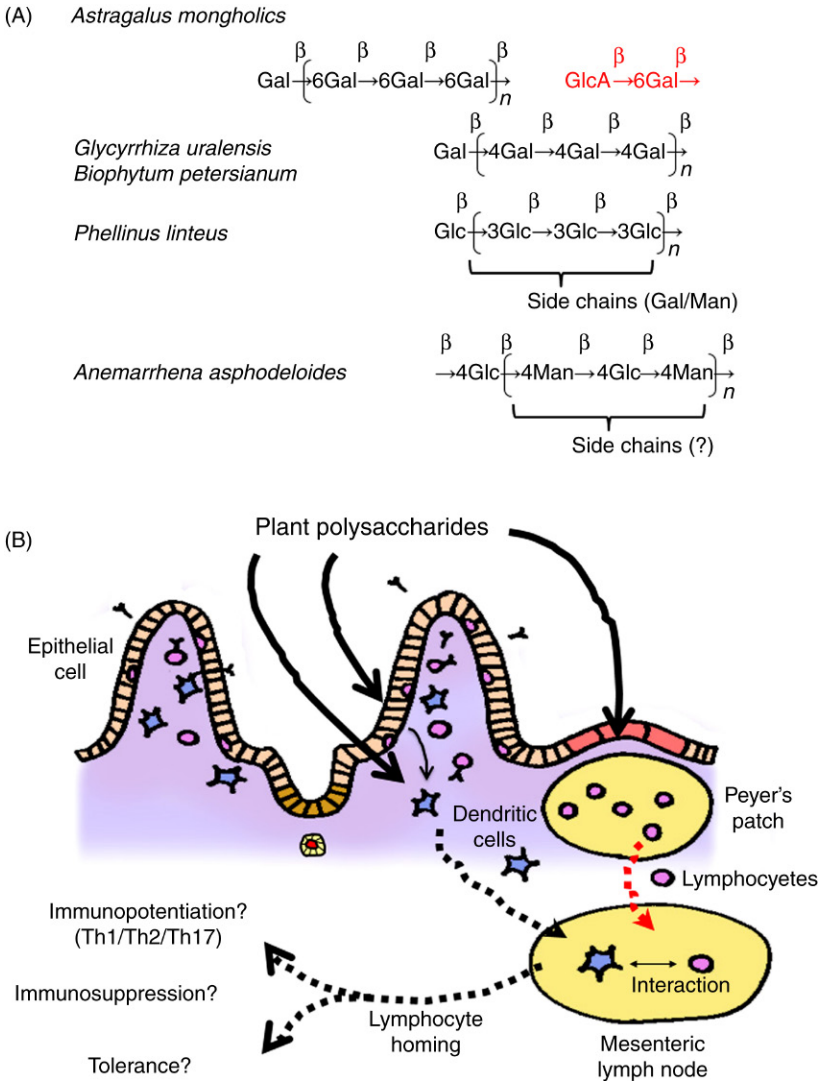


Figure 10.4: (A) Studied candidates of active carbohydrate chain units for Peyer's patch immunomodulation. (B) Hypothesized action mechanism of plant polysaccharides for immunomodulation through the intestinal immune system.

directed to immunopotentiation or immunosuppression through induction of effector and regulatory T-cells and B-cells. The potential therapeutic effect of this kind of polysaccharide immunomodulation is suggested in the next sections.

3.1.3 Combinatorial effects of immunomodulation with herbal polysaccharides

For further elucidation of immunomodulating potential of acidic herbal polysaccharides and their therapeutic perspectives were conducted numerous other in vitro and in vivo studies.

Kratchanova et al. (2010b) studied immunomodulating potential of water-extractable and acid-extractable pectic polysaccharides from the widely consumed leek (*Allium porrum*). The survival rate of mice pretreated with 2 mg/kg BW (i.p.) of two polysaccharides and subsequently infected with *Salmonella enteritidis* increased to 17.2 days in the case of water-extractable pectin, and to 10.5 days in the case of acid-extractable pectin from leeks, as compared with the survival rate of 5.5 days for control mice. Treating the mice with pectins led to an increase in the functional activity of their peritoneal macrophages and killing effect. Both polysaccharides activated complement system through classical and alternative pathways, as water-extractable was much more efficient. Additionally, Nikolova et al. (2013) showed that leek pectins could modulate ROS and RNS production from phagocytes in vitro and ex vivo. Yang et al. (2010) demonstrated that oral administration of herbal polysaccharides (LLPs) from *Lycopus lucidus* (50, 100, and 300 mg/kg) significantly enhanced the plaque-forming cells, serum hemolysin level, and delayed-type hypersensitivity (DTH) in response to antigen sheep red blood cells (SRBC) in a dose-dependent manner in BALB/c mice. In LLP-treated mice, phagocytosis capacity and concanavalin A-induced splenocyte proliferation were remarkably increased. Both higher doses of LLPs also caused a significant increase in the indices of thymus and spleen organs of mice. These findings show that *L. lucidus* polysaccharides could initiate nonspecific and specific immune-enhancing activities in vivo. Holderness et al. (2011) reported that acai fruit polysaccharides induced innate immune responses in human, mouse, and bovine peripheral blood mononuclear cells (PBMC). In mice, acai-PS incited neutrophil recruitment to the peritoneum and lung, as well as activated dendritic cells and macrophages in the lung after peritoneal injection and intratracheal administration. They induced dose-dependent IL-12 production within the lung, indicating proinflammatory Th1 response. These therapeutic effects show a potential use for acai polysaccharides in asthma and infectious disease. Kuang et al. (2011) studied the inhibitory effect of acidic polysaccharide from *Ephedra sinica* on DNFB-induced DTH in animal model in vivo. They showed that at a low dose of 50 mg/kg BW of ESP-B4 fraction ear tissue swelling and inflammatory cell infiltration were reduced, while in the high dose of 200 mg/kg BW tissue hypertrophy was reduced much obviously and a few inflammatory cell infiltration was presented. Furthermore, ESP-B4 in the higher dose decreased IL-2 and IL-4 serum levels and effectively suppressed $CD4^+/CD8^+$ ratio in serum of T-cell-mediated DTH mice. This shows immunosuppressive effects of studied polysaccharides and their potential use in the pathogenesis of T-cell-mediated autoimmune diseases and chronic inflammatory disorders. Liu et al. (2011) reported that *Astragalus* polysaccharides induced differentiation of splenic dendritic cells to IL-12-producing $CD11C^{high}$, $CD45RG^{low}$ dendritic cells, thus mediating activation of T-lymphocyte immune function and shifting of Th2 to Th1 response via IL-12. Therefore, *Astragalus* polysaccharides can regulate T-cell-mediated immunity by activation of dendritic cells, such as promoting cellular immunity and improving the immune functions in immunosuppression. Inngjerdingen et al. (2013) demonstrated that pectic fractions from *B. petersianum*, *Opilia celtidifolia*, and *Cola cordifolia* with complement fixation activity protected against *Streptococcus pneumoniae*

in a mouse pneumococcal infection model. This means that in vitro complement system activation led to an antibacterial protective effect in vivo. Kouakou et al. (2013) reported that polysaccharides from the leaves of *Clerodendrum splendens* exhibited beneficial effects in experimental autoimmune encephalomyelitis (EAE) in mice. One of the most efficient was pectic PS subfraction CSP-AU1 containing AG II. This subfraction-induced NO and cytokine production by human PBMC and monocyte/macrophages. CSP-AU1 treatment of animals with EAE (50 mg/kg BW, i.p., daily, 13 days) resulted in significantly reduced disease severity in EAE mice. Levels of IL-13, TNF-α, IFN-γ, IL-17, and GM-CSF were also significantly decreased, whereas TGF-β was increased in LN cells from CSP-AU1-treated EAE mice. The authors explained that CSP-AU1 stimulation of the innate immune system via TLR4 led to the induction of immunosuppression in EAE mice, which could be clinically beneficial effect of *Clerodendrum* polysaccharides on multiple sclerosis. Li et al. (2014b) purified RG II (KMPS-2E) from the roots of *Aconitum coreanum* that inhibited iNOS, TLR4, phospho-NF-*k*B–p65, phosphor-IKK, phosphor-IkB-α expressions, as well as the degradation of IkB-α and the gene expression of inflammatory cytokines, mediated by the NF-*k*B signal pathways in murine macrophages (50, 100, and 200 mg/mL). In rats, KMPS-2E (200 mg/kg BW) significantly inhibited carrageenan-induced paw edema as ibuprofen (200 mg/kg) within 3 h after a single oral dose. Polysaccharides are potent antiinflammatory constituents in *A. coreanum* roots and they have potential to aid inhibition of acute inflammation and also perspectives to be included in human diet for such purposes. Wang et al. (2015) obtained from the roots of *Panax quinquefolius* three acidic heteropolysaccharides with chemical characteristics of pectins and acidic hemicelluloses that upregulated macrophage production of NO, TNF-α, and IL-6 at 50 and 250 μg/mL in vitro.

Therapeutic potential of immunomodulating herbal polysaccharides could be also expanded to cardiovascular and liver diseases. Wang et al. (2014) showed that polysaccharides from selenium-enriched Ziyang green tea ZTPs displayed noteworthy scavenging effects against DPPH·, OH·, and O_2^-. The high in vitro antioxidant activity was further verified by suppressing CCl_4-induced oxidative liver damage in mice at 100, 200, and 400 mg/kg BW. Administration of ZTPs in mice prior to CCl_4 significantly prevented the CCl_4-induced increase in serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), and lactic dehydrogenase, as well as hepatic malondialdehyde (MDA) levels. Mice treated with ZTPs showed normal glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) activities, relative to CCl_4-treated group. Therapeutic potential of green tea polysaccharides could be related to hepatoprotective activity in preventing oxidative stress-related hepatotoxicity in liver. This study helps in the elucidation of therapeutic effects of the most consumed drink in the world, showing that polysaccharides are the essential functional ingredient of green tea, and its related nutraceutical products. Xie et al. (2015) extracted pectic polysaccharides (CASPs) from the roots of *Acanthioanax senticosus* that improved the symptoms of cerebral ischemia–reperfusion injury, and reduced the brain infarct volume and brain water content in Wistar male rats. In addition, CASPs elevated

SOD and GSH-Px activities and IL-10 levels, and reduced MDA, IL-1β, and TNF-α levels in the brain tissue of examined rats. The protective effect may be related to the improvement of CASPs on the antioxidant capacity of brain tissue and the inhibition of overproduction of inflammatory cytokines. Zhang et al. (2016) isolated a novel alkali-soluble acidic polysaccharide from Radix Polygoni Multiflori that exhibited significant immunomodulating potential by activating in vitro splenocytes and peritoneal macrophages. It also protected these immunocytes against immunosuppression induced by the anticancer drug 5-fluorouracil (5-FU). Rösch (2016) reported a novel approach, using mouse bone-marrow derived dendritic cells from TLR2/4 KO mice for evaluation of immunomodulatory properties of dietary fibers without interference from immunoactive LPS. They found that linear sugar beet pectic arabinan induced higher cytokine secretion than branched one. The authors concluded that pectic arabinans could modulate immune responses through C-type lectin receptors on immunocompetent cells, recognizing specific structural motifs.

3.2 Antitumor Activity Via Immune System Stimulation

Proposed therapeutic perspective of herbal polysaccharides, activating complement system and immune cells is expression of tumor-preventing, antitumor, and antimetastasis activities (Yamada and Kiyohara, 2007). For example, Mueller and Anderer (1990) showed that pectic RG, isolated from commercial *V. album* extract was able to enhance NK cytotoxicity of human PBMC by increasing formation of NK-cell/K562 tumor cell conjugation. The mechanism involves bridging of the NK and tumor cells by the active acetylated RG as follows: terminal acetylated Rha binds only to NK cells and on the other hand, terminal GalA binds only to tumor cells. Mistletoe was first proposed for treatment of cancer by Rudolph Steiner in 1920.

Similarly, nettle root extract is recommended for complaints associated with benign prostatic hyperplasia (BPH). The first clinical study was conducted in 1950 by Rückle, who showed that nettle root tea has a positive effect on patients with prostatic problems. More than 40,000 men suffering from BPH have been treated with various nettle root preparations. Usually, daily doses of 4–6 g as an infusion or 300–600 mg for 20% methanolic extract are applied in clinical studies. Twenty percent of methanolic extract has already proven its efficacy in BPH patients and is widely used in drug formulations (Chrubasik et al., 2007). Lichius et al. (1999) found that polysaccharide fraction POLY-M was one of the most effective constituents in 20% methanolic extract for inhibiting experimentally induced prostatic hyperplasia in mice. POLY-M was a mixture of glucans and pectic RG I (Wagner et al., 1994). Bazoton is a commercial drug on the basis of 20% methanolic extract from nettle root that is applied to treat prostate cancer and it contains about 1.7% polysaccharides. It is tested for acute toxicity, mutagenic and carcinogenic effects, as LD_{50} value for oral administration of Bazoton is greater than 30 g/kg in rats (Chrubasik et al., 2007). For comparison, in our two-stage screening study for immunomodulating acidic polysaccharides from 34 traditional European

medicinal plants, we found that crude nettle root polysaccharides expressed the most potent activity. Isolated rich in AG II pectic fraction was the most active constituent from the mixture against Peyer's patch cells (Georgiev et al., unpublished results). Neutral and pectic polysaccharides also stimulated ex vivo spontaneous ROS production in human neutrophils and in vitro NO production in murine macrophages (Georgiev et al., unpublished results).

Furthermore, it was found that commercial enzymatically or chemically produced modified citrus pectin (MCP) exhibits significant therapeutic effects on tumor metastasis and apoptosis in experimental animal models and in clinical trials (Eliaz et al., 2006). Monosaccharide and glycosidic linkage composition, defined molecular weight (10–20 kDa) and low degree of esterification (<10%) are key parameters for MCP to be able to enter blood circulation, and to bind cellular receptors on immunocompetent and tumor cells. It is considered that β-D-galactans in MCP are responsible for the activity (Morris et al., 2013). Beta-(1→4)-D-galactans interact with galectin-3 receptors on tumor cells through β-D-Gal*p* at O-3 and Arg144 from the receptor (Dumic et al., 2006). Generally, binding between pectic galactans and AGs to galectin-3 blocks tumor cell development, adhesion, and angiogenesis (Morris et al., 2013). In clinical trials with humans MCP PectaSol-C is administrated orally 3 times per day at a single dose of 5 g. The same dosage is recommended 1 week before surgery and between 2 and 4 weeks after the operation. Between 3 and 5 g/day are recommended for preventive therapy, but this is not clinically proved. MCP has a GRAS status and observed side effects in humans are loose stool and gastrointestinal discomfort (Eliaz et al., 2006). Interestingly, Courts (2013) found that unlike polyuronides, short-chain galactans and AGs derived from MCP were absorbed through an immobilized on porous membrane monolayer of tumor cells, inducing colon cancer from tumor cell line caco-2 (HTB-37). According to Niture and Refai (2013), 50 independent studies have demonstrated that MCP interacts with galectin-3 and inhibits tumor growth or cancer metastasis in vivo and in vitro in various types of cancers, including prostate, colon, breast, melanoma, and multiple myeloma. Currently, a clinical trial in Israel with duration 2013–17, entitled "Effect of MCP on PSA kinetics in biochemical relapsed PC with serial increases in PSA" is conducted with prostate cancer patients. The study is performed with commercial MCP PectaSol-C in a daily dose of 4.8 g in 6 capsules, 3 times per day.

Mistletoe, nettle root extracts, and MCP are classical examples for therapeutic efficacy of supplementation with polysaccharides. The next section presents some recent studies on perspective acidic herbal polysaccharides and their detailed therapeutic effects, examined predominantly in animal models.

Cao et al. (2010) demonstrated that treatment of S180 tumor-bearing mice with cyclophosphamid Cy (20 mg/kg/day), APS-3b, and APS-3c polysaccharide fractions from the roots of *A. sinensis* (100 mg/kg/day) increased their survival from 15.63 ± 0.68 days for the control to 19.38 ± 1.08, 22.75 ± 1.68, and 19.63 ± 1.12 days, respectively. Undoubtedly,

polysaccharides expressed lower activity than Cy, but appetite, activity, and coat luster of each animal in polysaccharide-treated groups were better. Additionally, polysaccharides increased the relative spleen and thymus weights. Polysaccharides stimulated NO production in peritoneal macrophages in vitro. The authors concluded that in vivo antitumor activity of APS-3b and APS-3c might be based on their T-lymphocyte activation via Th1 response and macrophage stimulation, rather than a direct cytocidal effect on the tumor cells. Lee et al. (2012) reported that an acidic *A. sinensis* polysaccharide possessed hematopoietic-enhancing and myeloprotective effects on human $CD34^+$ stem cells. They stated that it might be applied as a health-promoting agent for anemia patients and cancer patients under chemoradiation treatment. Xin et al. (2012) isolated two antitumor acidic polysaccharide fractions PTPa and PTPb (*Polygala tenuifolia*) with IC_{50} values of expressed cytotoxic activity against human lung adenocarcinoma cell line (A549) 47.8 and 41.2 µg/mL, respectively. For comparison, IC_{50} values for inhibition of human breast carcinoma (MCF7) and colon carcinoma (HT-29) were over 65 µg/mL for both polysaccharides. After daily i.p. administration of PTPa or PTPb to A549 tumor-bearing mice, the tumor volumes were statistically lower in polysaccharide-treated mice at low and high doses (50 and 100 mg/kg BW), compared with the control group. Treatment with two acidic polysaccharides caused an enhancement of SOD and catalase CAT activities in tumor-bearing mice and a reduction in thiobarbituric acid reactive substance (TBARS) level. The authors consider that it is reasonable to hypothesize that two polysaccharides prompt tumor regression at least partly through their antioxidative and radical scavenging properties. They found that initial PTP might express antitumor activity through mediation of GSH depletion and increase of intracellular ROS in cancer cells, thus resulting in apoptosis. In our in vitro study, we determined IC_{50} values for the cytotoxic activity of crude polysaccharide mixture from silver linden (flowers) linden PSC against A549 and HT-29 cell lines to 92.6 and 82.1 µg/mL (Georgiev et al., 2017a). At the same time linden polysaccharide fractions (free of LPS) obtained by ion-exchange chromatography stimulated in vitro NO production and corresponding iNOS expression in murine macrophages, and ex vivo ROS production in whole blood or isolated neutrophils at a concentration of 100 µg/mL (Georgiev et al., 2017b). Lin et al. (2013) isolated MDP-A1 and MDP-A2 acidic polysaccharide fractions with high GlcA content from the rhizoma of *Menispermum dauricum* that were able to inhibit cell proliferation of ovarian carcinoma SKOV3 cells. In addition, a significant reduction in tumor volume was observed in mice treated with doses of 25 and 50 mg/kg BW. Zhao et al. (2013) isolated antitumor acidic polysaccharide from *Portulaca oleracea* that inhibited in vitro HeLa cell proliferation and in vivo tumor growth (50–200 mg/kg BW) in U-14-bearing mice. The mechanism of antitumor activity was explained by sub-G1 phase cell cycle arrest, triggering DNA damage and inducing apoptosis. Similarly, in our in vitro study we obtained even higher cytotoxic effect of purslane polysaccharide mixture against HeLa cell line. Additionally, crude purslane polysaccharides purslane PSC stimulated human $CD4^+$ $CD25^+$ and $CD8^+$ $CD25^+$ T-cells and whole blood phagocytes ex vivo (Georgiev et al., 2017a).

Purslane is a widely distributed weed that is consumed in the Mediterranean and other countries and its polysaccharides are easily extracted with hot water in high yields and low content of LMWC. There is a perspective application of purslane polysaccharide-derived nutraceutical or food formulations for support of compromised immune status. Shu et al. (2015) found that supplementation (30 and 100 mg/kg BW) of Kunming mice bearing H22 ascitic hepatoma cells with a pectic polysaccharide (PSSC) from *Salvia chinensis* led to a decrease of tumor size and did not negatively influenced mouse body weight. Unlike cisplatin therapy (5 mg/kg BW) downregulated both parameters. Strong toxic impact of cisplatin on mouse immune system was shown by dramatic decrease of the amount of white blood cells in the blood, increased serum activities of AST and ALT, and serum levels of BUN and CRE. In parallel, polysaccharide-receiving mice did not show such changes. The polysaccharide elevated $CD4^+$ T-cell subpopulation in spleen and lymph nodes, increased cytotoxic activities of NK and $CD4^+$ T-cells, as all were compromised from the tumor implementation. Serum levels of IFN-$\gamma$and IL-2 increased and both IL-4 and 10 decreased by the polysaccharide administration. The authors concluded that suppressing tumor transplantation-induced PGE2 production was the key event mediating the antitumor immunostimulatory activity of the polysaccharide. The observed therapeutic effect initiates use of PSSC as a potential adjuvant reagent in the stimulation of an anticancer immune response in clinical HCC treatment.

3.3 Gastroprotective Effects Via Immune System Modulation

Still, oral administration of drugs remains the most convenient method for drug intake with the highest compliance rates among patients. Herbal polysaccharides from teas, folk remedies, functional drinks, and nutraceuticals pass easily through the GIT. That is why positive therapeutic effects of polysaccharides in the GIT are a direct result of their physicochemical nature and direct communication between their bioactive epitopes and immunocompetent cell receptors (Figs. 10.4A and B). Polysaccharides can be useful in all GID and corresponding diseases, such as inflammatory bowel disease (IBD). About 1.4 million people in the USA and 1 million in the EU suffer from IBD and the number of cases increases every year. Infiltration of inflammatory cells in the intestinal mucosa, disturbance of intestinal epithelium homeostasis, and abnormal production of proinflammatory and antiinflammatory cytokines lead to this inflammation. Crohn's disease and ulcerative colitis are examples of IBD that could lead to anemia, malnutrition, salt imbalance, and higher risk of colorectal cancer development (Hur et al., 2012). Polysaccharides are natural compounds that have already proven their efficacy in treatment and prevention of GID and could be used as part of the supplementation therapy and functional nutrition. Gastroprotective action of acidic polysaccharides is simply expressed by their antiulcer effect associated with protective adhesion to mucosal membranes, inhibition of hydrochloric acid, and pepsin secretion, and by their protective scavenging effect (Yamada, 1996). Sakurai et al. (1998) characterized with enzymes the antigenic epitopes in antiulcer pectic polysaccharides from *B. falcatum*.

They suggested that 6-linked galactosyl chains containing terminal GlcA or 4-O-Me-GlcA, attached to (1→3)-β-D-galactosyl chains, are important sugar residues in the antigenic epitopes of the ramified region of bupleuran 2IIc. Furthermore, acidic polysaccharides could exhibit a prebiotic effect, which influences intestinal microflora that has a vital role for IBD pathology (Xu et al., 2013). Thus, pectins and glucuronoarabinoxylans could be useful for prevention and treatment of IBD.

Cipriani et al. (2008) isolated two acidic heteroxylans with antiulcer activity from *Maytenus ilicifolia* and *Phyllanthus niruri* that are traditionally used for preparation of medicinal pills and teas for treating and preventing gastric ulcers. The polysaccharides reduced gastric lesions, induced by ethanol in Wistar rats by 65% and 78%, with ED_{50} = 40.0 and 20.4 mg/kg, each respectively. Later, Cipriani et al. (2009) demonstrated that polygalacturonic acid from the leaves of *M. ilicifolia* inhibited ethanol-induced gastric lesions in rats, with an ED_{50} of 103 mg/kg BW, suggesting that it had a protective antiulcer effect. Baek et al. (2010) showed that ginseng pectic polysaccharides expressed antirotavirus effect against human rotavirus HRV-Wa in an in vitro model with MA-104 cells. The authors stated that HG itself did not show an antirotavirus effect, so hairy regions might be the functional sites for expressed activity. These findings suggest that ginseng water extracts or polysaccharides are viable for prevention in rotavirus-induced diarrheal disease. Tanaka et al. (2010) demonstrated that RG with AG I side chains from the stems of cactus *Cereus peruvianus* significantly inhibited ethanol-induced gastric lesions in rats at an ED_{50} of 49 mg/kg BW. The cactus polysaccharide showed comparable activity with the positive control omeprazole (40 mg/kg BW, p.o.), but at a lower concentration of 30 mg/kg, revealing its potential as a phytotherapic drug. Austarheim et al. (2012b) reported antiulcer pectic polysaccharides from the leaves and bark of Malian plant *C. cordifolia*. The oral administration of 50 and 200 mg/kg BW of CCbark50 and CCleaf50 fractions 1 h before administration of 90% EtOH significantly inhibited ulcer formation in rats in a dose-dependent manner. Additionally, CCbark50 expressed a high complement fixation activity in vitro. Nascimento et al. (2013) showed that a pectic polysaccharide from the leaves of *Acmella oleracea* significantly inhibited ethanol-induced gastric ulcers in rats with an ED_{50} of 1.5 mg/kg BW. The authors mentioned that *A. oleracea*, locally known as jambu, is widely used in Legal Amazon in local dishes and in folk medicine. Simas-Tosin et al. (2014) reported that a glucuronoarabinoxylan (CNAL) from *Cocos nucifera* gum exudates reduced gastric hemorrhagic lesions, when orally administered (1 and 3 mg/kg BW) to rats prior to ethanol administration. The positive control treated with omeprazole (40 mg/kg BW, p.o.), also inhibited the gastric lesion area by 67%, which was similar to the inhibition observed for the dose of 1 mg/kg BW of CNAL. GAXs are around 80% of the gum exudate from coconut palm weight and are perspective candidates from technological and therapeutic point of view. Yang et al. (2014) found that polysaccharide fraction APS from *Astragalus membranaceus* (200 mg/kg BW) and dexamethasone (0.3 mg/kg BW) significantly downregulated TNF-α and IL-1β expressions,

and upregulated NFATc4 mRNA and protein expression in TNBS-induced experimental colitis in rats. Li et al. (2014a) demonstrated that purslane polysaccharides at doses 200, 400, and 800 mg/kg BW increased body weight, peripheral white blood cells, thymus, and spleen indexes, splenocyte proliferation and serum cytokines like IL-2, IL-4, and TNF-α in tumor-bearing rats. Furthermore, treatment with polysaccharides provided a dose-dependent protection against gastric cancer-induced oxidative injury by enhancing SOD, CAT, GSH-Px activities in rats. Thöle et al. (2015) revealed that acetylated RGs I from immature fruits of okra *Abelmoschus esculentus* exhibited antiadhesive activity against *Helicobacter pylori* to human gastric epithelial AGS cells in vitro. HBSS fraction inhibited more than 60% adhesion of three clinical strains (P12, 26695, TN-2) after preincubation at 1.0 mg/mL. They proved that methyl and acetyl esters were very important for the expressed antiadhesive activity. The inhibition mechanism was demonstrated by interaction of okra polysaccharides with several bacterial adhesins. Scientists concluded that okra water extract and isolated pectins might be interesting for development of antiadhesive products for establishment of preventive strategies against stomach infections caused by *H. pylori*. These products could be directly applied for prevention of early infections in children and during antibiotic eradication therapy. Harsha et al. (2016) demonstrated that pectic polysaccharide fraction MTrPP from *Curcuma longa* showed potent anti-*H. pylori* activity with IC_{50} value of 44 µg/mL, which was comparable to that of amoxicillin (26 µg/mL). The same fraction expressed anti-*H. pylori*-mucoadhesive property with IC_{50} of 36 µg/mL. MTrPP inhibited ulcer scores in a stress-induced gastric model in rats with rate up to 84% at 200 mg/kg BW, which was on par with the protection offered by lansoprazole (86%) at 50 mg/kg BW. The stomachs of rats pretreated orally with MTrPP significantly attenuated the process of ulceration. MTrPP could recover and prevent gastric mucin damage up to 87%, furthermore nearly 78% of increased H^+,K^+,-ATPase activity was normalized by its administration in ulcerative rats. Additionally, polysaccharide normalized TBARS, SOD, GSH-Px, and CAT levels. There were not any toxic effects observed. The preventive therapeutic effect of pectic polysaccharides on gastric ulcers was expressed by inhibition of excess acid production, maintenance of the gastric mucus layer, modulation of antioxidant mechanisms, and inhibitions of *H. pylori* growth and adherence.

3.3.1 Prebiotic effect

Immunomodulating acidic polysaccharides often possess prebiotic properties on the essential for gastrointestinal health and for the maintenance of human homeostasis lactobacilli and bifidobacteria (Aachary and Prapulla, 2011; Hartog et al., 2015). Intestinal microflora metabolizes polysaccharides to SCFA like acetic, propionic and butyric acids. SCFA play an important role in maintaining the function of epithelium barrier, regulation of epithelium proliferation, modulation of immune responses and prevention from colorectal cancer (Xu et al., 2013). For example, IBD patients have lower levels of SCFA, which is a result from the change in the comensal bacterial populations presented by increased number of proteobacteria, actinobacteria, and decreased populations of Firmicutes and Bacteroidetes.

IBD patients suffer from lack of bifidobacteria as well. Normalized dietary fiber-rich diet exhibits potential preventive effects against GID. Plant polysaccharides are assimilated to different extent from residential microflora, as increasing the complexity leads to more difficult degradation and lower SCFA production. Generally, amylopectin, β-glucans, and inulins are much better prebiotics than pectins. It is very important to reveal the relation between the structural features of different polysaccharides and the selective growth of microbial populations (Xu et al., 2013). On therapeutic point of view, this is important for development of polysaccharide formulations for manipulation of microbial flora and production of specific bioactive metabolites.

Yang et al. (2012) demonstrated that an aqueous extract (SAEAF) and pectic AG II (AFP) from *Anoectochilus formosanus* expressed prebiotic activity in vitro and in vivo. SAEAF exhibited prebiotic effects, including a decrease in cecum pH and increase of calcium absorption and fecal bifidobacteria after 4-week oral administration of 200 and 400 mg/kg BW in growing rats. Ethanol-precipitated AFCP showed effective promotion of *B. breve* growth. AFCP treated with enzymes to remove starch content provided AFP, which markedly enhanced *B. breve* proliferation. Mice receiving an AFP dosage of 20 mg/kg BW produced an increased number of *Bifidobacterium* colonies in the stool on day 3. In RT-PCR analysis of *Bifidobacterium*, AFP increased the expression of β-galactosidase, α-L-arabinofuranosidase and ABC transporter related to nutrient uptake. Compared to the same dose of inulin, AFP presented a higher prebiotic index (PI). Min et al. (2014) found that colon index in Kunming mice supplemented with 0.15, 0.3, and 0.6 mg/g BW crude acidic polysaccharide from *Cyclocarya paliurus* leaves significantly increased, as the administration time increased and the length of colon in the polysaccharide group was longer than the control. The pH values in caecum, colon, and feces of the polysaccharide group were significantly reduced. Furthermore, the levels of SCFA in caecum, colon and feces of mice fed with 0.6 mg/g BW significantly increased. Khodaei et al. (2016) found that enzyme-released galactooligosaccharides and oligomers from potato RG I were fermented by *Bifidobacterium* and *Lactobacillus* species with higher selectivity than the initial polymer. Potato RG I and its corresponding oligosaccharides/oligomers did not have a negative effect on Bacteroidetes and seem to reduce the population of the *C. leptum*. RG I-based oligomers and galactooligosaccharides exhibited similar PI to FOS. Total amounts of SCFAs, generated upon the fermentation of RG I oligomers and galactooligosaccharides were higher than those obtained with RG I and FOS. Therefore, complex acidic polysaccharides are difficult for microbial degradation but their enzyme-derived oligosaccharides are perspective prebiotics. Similarly to pectic oligosaccharides, hemicellulosic xylooligosaccharides, and arabinoxylan oligosaccharides show promising results (Aachary and Prapulla, 2011; Neyrinck et al., 2012).

Table 10.1 summarizes the results on in vivo experiments for evaluation of perspective therapeutic effects of herbal acidic polysaccharide presented earlier.

Table 10.1: In vivo evaluation of perspective therapeutic effects of herbal acidic heteropolysaccharides.

Medicinal Plants	Polysaccharide	Treatment (Daily Dose)	Effects	References
In vivo combinatorial effects of immunomodulation with herbal polysaccharides				
A. porrum, aerial part	Water- and acid-extractable pectic polysaccharides	2 mg/kg BW (i.p.)	Increased survival rate of *S. enteritidis* infected mice.	Kratchanova et al. (2010b)
L. lucidus, leaves	Water-extractable pectic polysaccharides	50, 100, and 300 mg/kg BW (p.o.)	Enhanced phagocytosis capacity, serum hemolysin level, and DTH in response to antigen SRBC; increase in the indices of thymus and spleen organs.	Yang et al. (2010)
Acai fruit	Water-extractable pectic polysaccharides	5 and 50 µg (i.p.); 1.56–500 µg (i.t.)	Neutrophil, macrophage, and dendritic cell activation; proinflammatory Th1 response in lungs.	Holderness et al. (2011)
E. sinica, stems	Water-extractable acidic polysaccharide	50 and 2000 mg/kg BW (p.o.)	Immunosuppressive effects on DNFB-induced DTH in mice.	Kuang et al. (2011)
P. ginseng, leaves	Water-extractable Rhamnogalacturonan II	20 mg/kg BW (i.p.)	Suppressed asthmatic reaction in an ovalbumin-induced murine model of asthma.	Jung et al. (2012)
B. petersianum; aerial parts; *O. celtidifolia*, leaves; and *C. cordifolia*, bark	Water-extractable pectic polysaccharides	125 and 25 µg (i.p.)	Protective effect in *S. pneumonia* infected mice.	Inngjerdingen et al. (2013)
C. splendens, leaves	Water-extractable pectic polysaccharides	50 mg/kg BW (i.p.)	Significant reduction of disease severity in EAE mice.	Kouakou et al. (2013)
A. coreanum, roots	Water-soluble rhamnogalacturonan II	200 mg/kg BW (p.o.)	Significantly inhibited carrageenan-induced paw edema in rats.	Li et al. (2014b)
Ziyang green tea	Water-extractable acidic polysaccharides	100, 200, and 400 mg/kg BW (p.o.)	Protective effect on the CCl_4-induced oxidative damage in mice.	Wang et al. (2014)
A. senticosus, roots	Water-extractable pectic polysaccharides	50, 100, and 200 mg/kg BW (i.g.)	Protective effects in cerebral ischemia-reperfusion injury in rats.	Xie et al. (2015)
Antitumor activity via immune system stimulation				
Nettle root	Water infusion or 20% methanolic extract, containing glucans and pectic rhamnogalacturonans	4–6 g infusion or 300–600 mg, 20% methanol extract	Effective in benign prostatic hyperplasia.	Chrubasik et al. (2007)

Citrus fruit	Modified citrus pectin (PectaSol-C)	4.8 g (p.o.)	Determination of effectiveness in improvement of PSA kinetics in men with biochemical relapsed prostate cancer and serial increases in PSA levels men with prostate cancer. Documentation of side effects.	Running clinical trial 2013–17, Institute of Oncology, Meir Medical Center, Kfar-Saba, Israel
A. sinensis, roots	Water-extractable acidic polysaccharides	100 mg/kg BW (i.p.)	Antitumor activity might be based on their T-lymphocyte activation via Th1 response and macrophage stimulation in S180 tumor-bearing mice.	Cao et al. (2010)
P. tenuifolia, roots	Acidic polysaccharides	50 and 100 mg/kg BW (i.p.)	Might express tumor regression at least partly through their antioxidative and radical scavenging properties in A549 tumor-bearing mice.	Xin et al. (2012)
M. dauricum, rhizoma	Water-extractable GlcA-rich polysaccharides	25 and 50 mg/kg BW (i.p.)	Significant reduction in tumor volume in SKOV3 carcinoma-bearing mice.	Lin et al. (2013)
P. oleracea, aerial part	Water-extractable acidic and neutral polysaccharides	50–200 mg/kg BW (p.o.)	Antitumor activity was explained by sub-G1 phase cell cycle arrest, triggering DNA damage, and inducing apoptosis in U-14-bearing mice.	Zhao et al. (2013)
S. chinensis, aerial part	Water-extractable pectic polysaccharide	30 and 100 mg/kg BW (i.p.)	Antitumor activity through augmentation the cytotoxic activity of NK and $CD8^+$ T cells and activity of $CD4^+$ T cells in mice bearing H22 ascitic hepatoma cells.	Shu et al. (2015)
Gastroprotective effects via immune system modulation				
M. ilicifolia, leaves and *P. niruri*, whole plant	Alkali-extractable acidic heteroxylans	ED_{50} = 40.0 and 20.4 mg/kg BW (p.o.)	Reduction of ethanol-induced gastric lesions in rats.	Cipriani et al. (2008)
M. ilicifolia, leaves	Water-extractable polygalacturonic acid	ED_{50} of 103 mg/kg BW (p.o.)	Protective antiulcer effect in ethanol-induced gastric lesions in rats.	Cipriani et al. (2009)
C. peruvianus, stems	Water-extractable rhamnogalacturonan I	ED_{50} of 49 mg/kg BW (p.o.)	Significantly inhibited ethanol-induced gastric lesions in rats.	Tanaka et al. (2010)
C. cordifolia, bark, leaves	Water-extractable pectic polysaccharides	50 and 200 mg/kg BW (p.o.)	Significantly inhibited ulcer formation in treated with 90% ethanol rats.	Austarheim et al. (2012a,b)

(*Continued*)

Table 10.1: In vivo evaluation of perspective therapeutic effects of herbal acidic heteropolysaccharides. (*cont.*)

Medicinal Plants	Polysaccharide	Treatment (Daily Dose)	Effects	References
A. oleracea, leaves	Water-extractable pectic polysaccharide	ED_{50} of 1.5 mg/kg (p.o.)	Significantly inhibited ethanol-induced gastric ulcers in rats.	Nascimento et al. (2013)
C. nucifera, gum exudates	Alkali-extractable glucuronoarabinoxylan	1 and 3 mg/kg BW (p.o.)	Reducing gastric hemorrhagic lesions in rats.	Simas-Tosin et al. (2014)
A. membranaceus	Polysaccharide mixture	200 mg/kg BW (p.o.)	Significantly downregulated TNF-α and IL-1β expressions, and upregulated NFATc4 mRNA and protein expression in TNBS-induced experimental colitis in rats.	Yang et al. (2014)
P. oleracea, aerial part	Water-extractable polysaccharides mixture	200, 400, and 800 mg/kg BW (p.o.)	Antioxidant and immune-enhancing activities against experimentally induced gastric cancer in rats.	Li et al. (2014a)
C. paliurus, leaves	Water-extractable acidic polysaccharide	0.15, 0.3, and 0.6 mg/g BW (p.o.)	Increased colon index, lowering pH, improving faecal moisture, and increasing SCFAs level in mice.	Min et al. (2014)
C. longa, roots	Ammonium oxalate-extractable pectic polysaccharide	200 mg/kg BW (p.o.)	Inhibition of excess acid production, maintenance of gastric mucus layer, modulation of antioxidant mechanisms in gastric ulcer rats, and in vitro inhibitions of *H. pylori* growth and adherence.	Harsha et al. (2016)
A. formosanus	Water-extractable pectic arabinogalactan II	20 mg/kg BW (p.o.)	Produced an increased number of *Bifidobacterium* colonies in the mice stool on day 3 and in vitro increased expression of their ABC transporter related to nutrient uptake.	Yang et al. (2012)

3.3.2 Unique target-specific drug delivery

Herbal polysaccharides expressing good jellifying properties could be used as target-specific carriers of different drugs in GIT, which is a promising application in drug formulations. For instance, Perera et al. (2010) tested in vitro novel pectin–4-aminothiophenol conjugate microparticles for colon-specific drug delivery of metronidazole that is used in various colonic diseases like Crohn's disease, amoebiasis, and diverticulitis. A 34.4-fold increased amount of metronidazole was retarded within Pec–ATP microparticles when they were exposed to simulated conditions of the small intestine. A rapid release of metronidazole from Pec–ATP microparticles was demonstrated by microbial pectinase degradation. Das et al. (2011) reported an optimized Zn-pectin-chitosan formulation for colonic-specific delivery of resveratrol in an in vivo rat model. They suggested that this optimized composite could be useful for further in vivo colitis or colorectal cancer disease studies. Elyagoby et al. (2013) developed zinc pectinate pellets for colon-specific delivery of the anticancer drug 5-FU. They showed in a rat model that less than 25% of the drug was released at the upper GIT but the majority of it was released upon prolonged dissolution and in response to colonic microbial pectinase. In vivo analysis indicated that a remarkably larger fraction of the drug was available in the colon when 5-FU was delivered by means of intracapsular coated pellets (322.18 ± 23.64 μg) instead of free drug (9.63 ± 4.10 μg). Furthermore, the colon-specific delivery of 5-FU could render a lower dose needed than that by injection route, which clearly shows feasibility of pectin in drug delivery systems. Villanova et al. (2015) reported chemically modified pectin with acrylic monomers for potential use as a mucoadhesive excipient for mucoadhesive drug delivery systems.

3.4 Perspective Therapeutic Effects of Polysaccharide Complexes

As mentioned earlier, acidic polysaccharides often present as bioactive complexes and glycoconjugates in herbal drinks, extracts and folk remedies. Interestingly, this kind of coupling with polyphenols, lignin, proteins, and minerals leads to formation of new immunoactive agents. In Table 10.2 results are summarized on in vivo experiments for evaluation of perspective therapeutic effects of polysaccharide complexes presented in the next section.

3.4.1 Polysaccharide-lignin conjugates

Kiyohara et al. (2000) demonstrated that water-soluble pectic LCC were involved in the in vitro and in vivo intestinal immune system modulation through murine Peyer's patch-mediated bone marrow cell-proliferation enhancing activity by the Kampo medicine formulation Juzentaihoto (Shi-Quan-Da-Bu-Tang in Chinese, TJ-48). Bone-marrow cell proliferation was used to estimate the relative production of hemopoietic growth factors from Peyer's patch cells. Later, the Japanese scientists found that similarly LCC were responsible for Peyer's patch-mediated bone marrow cell-proliferation enhancing activity of Hochuekkito (Bu-Zhong-Yi-Qi-Tang

Table 10.2: In vivo evaluation of perspective therapeutic effects of acidic herbal polysaccharide complexes.

Medicinal Plants	Polysaccharide	Treatment (Daily Dose)	Effects	References
Polysaccharide-lignin conjugates				
Kampo medicine formulation TJ-48, Tsumura & Co. (Tokyo, Japan)	Water-extractable pectic-polysaccharide-lignin	150 mg/kg BW (p.o.)	(1) Ex vivo murine Peyer's patch-mediated bone marrow cell-proliferation enhancing activity.	Kiyohara et al. (2000)
Kampo medicine formulation TJ-41, Tsumura & Co. (Tokyo, Japan)	Water-extractable pectic-polysaccharide-lignin	7.7 mg/kg BW (p.o.)	(1) +Enhanced influenza virus specific IgG antibody titer in sera of infected young mice.	Kiyohara et al. (2011)
Pinus densiflora, cones	Alkali-extractable LCC extract	1 mg tablets with vit. C or solution (p.o.)	Promoted faster healing, reduction of symptoms, and reducing the frequency of recurrence episodes in HSV-1 infection in patients.	Sakagami et al. (2010)
E. canadensis, flowering parts	Alkali-extractable pectic polyphenolic-polysaccharide conjugate	7–9 IU/mg of heparin standard (229.5 IU/mg)	Anticoagulant activity in rats.	Pawlaczyk et al. (2011)
M. chamomilla, flowering parts	Alkali-extractable polyphenolic-pectic polysaccharide conjugate	100 µg/mL	Ex vivo reduced platelet aggregation in platelet-rich plasma obtained from patients with cardiovascular disorders.	Bijak et al. (2013)
Polysaccharide-protein conjugates				
C. sinensis, low-grade green tea	Water- and alkali-extractable acidic polysaccharide-protein conjugates	150 mg/kg BW (p.o.)	Suppressed spontaneous diabetes mellitus in NOD mice.	Chen et al. (2010)
L. barbarum, fruit	Water-extractable polysaccharide-protein complex	100 µg/mL (i.p.)	Immunostimulatory activity predominantly on macrophages through CD86 and MHC-II expressions; induction of TNF-α and IL-1β production in macrophages.	Zhang et al. (2011)
S. canadensis, flowers	Alkali-extractable acidic polyphenolic–polysaccharide–protein	75 mg/kg BW (p.o.)	Antitussive activity in citric acid-induced coughs in guinea pigs.	Šutovská et al. (2013)
Polysaccharide-mineral complexes				
Se-enriched Ziyang green tea, Tea Industry Co., Ltd.	Water-extractable Se-polysaccharide complex	100, 200, and 400 mg/kg BW (p.o.)	Tumor regression in U-2 OS cancer xenograft model in BALB/c athymic mice.	Wang et al. (2013)
A. macrocephala	Selenylated polysaccharide	1 mg (i.m.)	Selenylation significantly increased immune-enhancing activity in chickens.	Liu et al. (2015a)

in Chinese, TJ-41). They obtained statistically significant positive correlation between enhancing activities of purified glycoconjugates against Peyer's patch immunocompetent cells and epithelial cells. TJ-41 formulation and its lignin-polysaccharide complex F-5 (7.7 mg/kg BW) succeeded to enhance influenza virus specific IgG antibody titer in sera of young mice (Kiyohara et al., 2011). In both studies, polysaccharides and lignin were important for the activity. The authors explained that TJ-48 and TJ-41 are clinically used in Japan to potentiate compromised immune status of patients with chronic diseases and after surgery. TJ-48 is useful formulation against tumor diseases and TJ-41 for respiratory diseases. Further, Jung et al. (2012) demonstrated that RG-II from *P. ginseng*, which is one of the constituents of TJ-41, suppressed asthmatic reaction in an ovalbumin-induced murine model of asthma. RG II reduced IL-4 production but increased INF-γproduction, and inhibited GATA-3 gene expression. RG-II also inhibited asthmatic reactions including an increase in the number of eosinophils in bronchoalveolar lavage fluid, an increase in inflammatory cell infiltration in lung tissues, airway luminal narrowing, and airway hyperresponsiveness. Sakagami et al. (2010) reviewed their studies on pharmacologically active LCC from pine and other sources, as summarized that they expressed antiviral, antimicrobial, antiparasite, antitumor, antimutagenic, and immunomodulating activities. They emphasized that phenylpropenoid polymer moiety of LCC has potent antiviral activity, whereas carbohydrate moiety has immunopotentiation activity, possibly via activation of TLR pathway and chemokine expression. They proved by radiolabeling that pine LCC was partly absorbed in the intestine and reached to blood and liver in experimental mice. A clinical trial with 48 patients, where evaluating anti-HSV-1 activity of a synergistic formulation between pine cone LCC and vitamin C was carried out, suggesting its possible applicability for the prevention and treatment of HSV-1 infection. Interestingly, Saluk-Juszczak et al. (2010) found that acidic polyphenolic-polysaccharide conjugates from six medicinal plants from Asteraceae family possessed antioxidative properties and protected ex vivo human platelet proteins against peroxynitrite toxicity. Pawlaczyk et al. (2011) reported a pectic polyphenolic-polysaccharide conjugate from *Erigeron canadensis* with in vivo anticoagulant and ex vivo antiplatelet activities. The most active anticoagulant fraction showed activity at 50 μg/mL, corresponding to 7–9 IU/mg of heparin standard (229.5 IU/mg), measured in a PTT test. The authors concluded that although this plant preparation had much lower anticoagulant activity than heparin, its antiplatelet activity might be an additional advantage in becoming an adjunctive agent in treatment like of deep vein thrombosis, especially of patients with some resistances on acetylsalicylic acid derivatives drugs. Bijak et al. (2013) showed that a pectic polyphenolic-polysaccharide conjugate from *Matricaria chamomilla* reduced platelet aggregation at a concentration of 100 μg/mL, induced by multiple agonists in platelet-rich plasma obtained from healthy donors and patients with cardiovascular disorders. Glycoconjugate did not show any in vitro cytotoxicity effects under the studied conditions. Blood platelets are the critical mediators of haemostasis and blood coagulation. They are implicated in the metastatic process of tumor cells, and in the pathogenesis of infection diseases. Anticoagulant activity of herbal polysaccharide conjugates expands their application potential.

Additionally, in our two-stage screening for immunomodulating polysaccharides from 34 traditional European medicinal plants through in vitro anticomplementary test and ex vivo Peyer's patch cell activation, we found that perspective complement immunomodulators were polysaccharides and polysaccharide-containing complexes from nettle (root and leaves), elecampane, thyme, purslane, soapwort, red oregano, bulhead (fruit), lady's mantle, lavender, verbena, linden, etc. (Georgiev et al., unpublished results). Polysaccharides are present in herbal teas and commercial water extracts, and must be partly responsible for their immunomodulating properties. Some polysaccharide complexes were rich in polyphenols and showed high antioxidant activity through ORAC and HORAC methods. The polysaccharide complexes from thyme, nettle leaves, red oregano, lavender, and tormentil were examples with high immunomodulating and antioxidant activities. The polysaccharide complexes contained not only polysaccharides and polyphenols. In our recent study on crude polysaccharides from linden, lavender, and purslane, we found that aside polysaccharides, polyphenols (phenolic acids, phenylpropanoids, etc.), saturated and unsaturated fatty acids, and higher paraffins presented in different amounts in the three samples on the basis of GC-MS and HPLC-UV analyses (Georgiev et al., unpublished results). The polysaccharides were responsible for expression of high anticomplementary activity through the classical and alternative pathways and polyphenols through the classical pathway. Further, rich in saturated fatty acids and hydrocarbons petroleum ether extracts showed weak activity. Linden butanolic extract expressed much lower anticomplementary activity through the classical pathway in comparison to lavender butanolic extract. It even showed antagonistic effect in combining with active extracts that was further explained by its high fatty acid content. After combination of SEC on Sephacryl S-200 and solid-phase extraction on Amberlite XAD7HP, we obtained potent chPS-L1 (free of LPS) pectic and chPP-L polyphenolic fractions that expressed IC_{50} values of 38.9 and 539.4 μg/mL or 94.0 and 86.9 μg/mL through the alternative or classical pathway, respectively. Thus, polysaccharides and polyphenols showed high anticomplementary activity. Their combined action deserves more scientific attention and possesses potential for further development of multifunctional nutraceuticals. Polysaccharides could serve as transporters and at the same time as protectors of polyphenols through the GIT to the colon, where they may express their antioxidant and different physiological effects (Jakobek, 2015). Polysaccharides and polyphenols could express protective activity against GIT inflammation and related disorders like IBD and colorectal cancer (Denev et al., 2014), and both positively influence lipid metabolism and neutralization of exogenous and endogenous toxicants in the human body (Groudeva et al., 1997; Stantschev et al., 1979; Yaneva et al., 2002). We have demonstrated that the addition of rosehip extract to chokeberry, blackberry, blackcurrants, or elderflower extracts led to a synergistic effect in ORAC antioxidant activity of the mixed extracts that reached up to 64%. The removing of rosehip polysaccharides inhibited the synergistic effect, indicating that rosehip pectic polysaccharides were at least partially involved in the observed synergism (Kratchanova et al., 2014). Herbal polysaccharides with considerable antioxidant and antiinflammation capacities have been shown to be beneficial in aging and age-related

neurodegenerative diseases (Li et al., 2014c). Polysaccharide-polyphenol complexes influence organoleptic qualities of foods and drinks as polysaccharides could prevent negative interactions between polyphenols and digestive enzymes (Jakobek, 2015). Practically, therapeutic combinatorial effect of polyphenols and polysaccharides has a great perspective in treatment of socially important diseases of our century, including cardiovascular, tumor diseases, and diabetes (Daskalova et al., 2015; Tomova et al., 1999).

3.4.2 Polysaccharide-protein conjugates

There are numerous studies on immunomodulating polysaccharide-protein conjugates and their perspective therapeutic effects (Nie and Xie, 2011). Particularly, antidiabetic potential of such glycoconjugates is very promising. For example, Chen et al. (2010) showed that acidic polysaccharide-protein conjugates from low-grade green tea could suppress spontaneous diabetes mellitus in NOD mice. Oral administration of 150 mg/kg BW TPC-W fraction significantly decreased the level of blood glucose and antiglutamic acid decarboxylase (GAD) antibody level in NOD mice. Lee et al. (2011) isolated three lignin-carbohydrate-protein complexes from seeds of *Pimpinella anisum* with in vitro antiviral activity against HSV-1 and 2, human cytomelagovirus and measles virus and in vitro immunostimulating activity against murine macrophages. Complexes were found to interfere with virus adsorption to the host cell surface and directly inactivate viruses. Zhang et al. (2011) reported that after digestion of protein moiety from the polysaccharide-protein complex LBPF4 from *Lycium barbarum* was obtained LBPF4-OL that lost activation ability on T-lymphocytes. LBPF4-OL expressed immunostimulatory activity predominantly on macrophages through CD86 and MHC-II expressions in vivo. It induced TNF-α and IL-1β production in a dose-dependent manner in macrophages. Additionally, B-cell proliferation was induced in the presence of macrophages. Šutovská et al. (2013) found that acidic polyphenolic–polysaccharide–protein complex from the flowers of *Solidago canadensis* expressed in vivo antitussive activity in citric acid-induced coughs in guinea pigs. Antitussive effect of the highest dose 75 mg/kg BW was 10% lower in comparison with that of codeine and it was without any side toxicity. Wang et al. (2015) isolated two potential prebiotic acidic polysaccharide-protein complexes (RP1 and RP2) from rapeseed that contained uronic acids and sulfates. Proliferative potency of crude polysaccharide, RP1 and RP2, against bifidopbacteria was 41.93, 68.15, and 60.08% of that of FOS. Clearly, the bacteria supplemented with RP1 and RP2 produced large amounts of organic acids, showing that both fractions were metabolized to some extent. Rapeseed is currently oilseed crop in Europe and this study opens some utilization possibilities of its waste.

3.4.3 Polysaccharide-mineral complexes

Herbal polysaccharides form complexes with minerals that also could potentiate and expand their immunomodulating activities. Wang et al. (2013) studied tumoricidal effect of a selenium-polysaccharide complex from Ziyang green tea on human osteosarcoma U-2 OS cells in vitro and in vivo in an animal model. Se-polysaccharide complex contained 94.5%

total sugars, 2.1% uronic acids, and 2.14 μg/g Se. In U-2 OS cancer xenograft model in BALB/c athymic mice, Se-ZYTP oral administration at three doses (100, 200, and 400 mg/kg daily for 28 days) resulted in obvious tumor regression, as compared to model control. The authors postulated that Se-polysaccharide complex might have cancer-preventive and cancer-therapeutic benefit for human osteosarcoma. Liu et al. (2015a) reported that selenylation of *A. macrocephala* polysaccharide AMP led to higher immune-enhancing activity in vitro and in vivo. In the in vivo test 14-day old chickens after vaccination with ND vaccine were intramuscularly injected with 1 mg $sAMP_9$ and AMP in solution, as lymphocyte proliferation rate in $sAMP_9$ group (59.63%) was significantly higher than those in AMP group (29.79%). Additionally, IFN-γ, IL-2, and IL-6 serum levels in experimental chickens treated with sAMP were higher than these with AMP or control groups. That is why selenylation significantly increased immune-enhancing activity of studied polysaccharides.

4 Preparation of Immunomodulating Acidic Heteropolysaccharides and Their Complexes

Some herbal polysaccharides are perspective immunomodulating agents for modern phytotherapy, functional and dietetic nutrition, but their profitable industrial purification and preservation of the natural formulation are still an open issue. The main problems are the low yields, expensive and exhaustive isolation, and purification procedures from herbal matrices. However, heparin is a good example for polysaccharide with unique biological properties that initiated its production from complex animal tissues, even on a pharmaceutical grade (Bhaskar et al., 2012). On the other hand, there are plenty of cheap industrial herbal residues that are waiting for a proper utilization, as especially attractive are by-products from cosmetic industry (Slavov et al., 2013, 2016). Therefore, it is clear that the most critical point is discovery of effective know-how purification technologies to attract industrial interest. That is why different immunoactive tea polysaccharide-containing complexes called polysaccharide extracts from traditionally used plants in the popular herbal medicine are currently produced. Interestingly, Corrêa-Ferreira et al. (2014) reported that traditional infusions from *Artemisia absinthium* and *A. vulgaris* contained 5.8 and 7.0% crude polysaccharide mixtures or 58 and 98 mg in each 200 mL infusion prepared from 1.0 and 1.4 g herbal material, respectively. Up to now clinically applied in Japan and China are mushroom β-glucan extracts (lentinan and schizophillan, registered as drugs in Japan), and pectic polysaccharide-containing extracts from *A. membranaceus* and *Achyranthes bidentata* (He et al., 2012). They could be referred to tea-like products. Nevertheless, this preparation technique may put in suspicion the therapeutic effect of the complex mixture and opens toxicological and adverse effect concerns. At the same time there is a Chinese *Astragalus* polysaccharide product (Jirek Animal Pharmacy Co., Ltd.) for intravenous use in animal health care. Undoubtedly, hot water is one of the best solvents for extraction of bioactive pectic polysaccharides and sometimes acids, alkali, or chelating agents are added to hot water for increasing

polysaccharide extractability or for sequential extraction of pectic and hemicellulosic polysaccharides. It is obvious that the addition of chemicals in water leads to essential changes in the structure and biofunctionality of polysaccharide macromolecule in comparison to hot water itself. Polysaccharides are separated from water extracts by alcohol precipitation, further fractionated and purified by ion exchange, size exclusion, and sometimes by affinity chromatography on a laboratory scale. Continuous boiling with water is used in the Japanese Kampo medicine, where decoctions are prepared from mixture of carefully selected herbs for specific health disorders and multiorgan targets. During preparation complicated ingredient interactions appeared and vital combinatorial pharmacological effects are reached. Therefore, synergistic or antagonistic effects occur, which are believed to be expressed in the potentiation of pharmacological effect of a single herb or a compound (changed extractability), reduction of particular adverse effects, and modulation of drug transport and metabolism (Yamada, 2005). Generally, hot-water extraction leads to extraction or formation of complex mixtures of different bioactive macromolecular and LMWC-acidic and neutral (hetero)polysaccharides (pectic polysaccharides, glucuronoarabinoxylans) and their conjugates with lignin, proteins and minerals (B, Se), as well as interacted physicochemically phenolic acids, flavonoids (catechins, tannins), saponins (triterpenic acids), fatty acids, hydrocarbons, phytosterols, and so on. Another preparation method, more applicable for glucuronoarabinoxylans and LCC, includes extraction with acidic solutions and/or diluted alkali. Sonication and microwave heating are also used to aid extraction of bioactive polysaccharides (Chen et al., 2016; Ebringerová and Hromádková, 2010; Liu et al., 2016; Zhu et al., 2016). Particularly, in our previous study microwave pretreatment of fresh orange peels led to considerable increase in pectin yield and quality (Kratchanova et al., 2004).

5 Concluding Remarks

Traditional therapeutic effects of herbs, such as immune potentiation, antiinflammatory, gastroprotective, and detoxifying actions could be partly explained by the presence of acidic heteropolysaccharides. Bioactive herbal pectic polysaccharides are easily coextracted with hot water, as is demonstrated by the industrially applied Kampo medicine technique. Arabinogalactan side chains contribute to the high carbohydrate-specific bioactivity of the whole molecule of herbal polysaccharides (Figs. 10.1A and 10.4A–B). This chapter shows that herbal pectins adequately contribute to both immune activation and immune suppression, when it is needed for helping in recovery and maintenance of homeostasis in living cells and whole organism. They could be useful in the treatment of immune disturbances like infectious and tumor diseases as immune system activators and for chronic inflammatory disorders like IBD (i.e., ulcerative colitis) and autoimmune diseases as immune system suppressors and regulators of oxidative enzymes. On the other side, bioactive glucuronoarabinoxylans that are generally extracted with diluted alkali have the potential to be used for prevention and treatment of gastric lesions. Additionally, it was

demonstrated that acidic polysaccharides could express prebiotic properties and also to be used for unique target-specific drug delivery in the GIT.

Acidic polysaccharides are often conjugated with noncarbohydrate polymers, (physico) chemically bounded with LMWC, and form complexes with inorganic elements. Interestingly, these kinds of coupling with polyphenols, lignin, proteins, and minerals lead to formation of new immunoactive agents. It was demonstrated that LCC express intestinal immunomodulating properties through Peyer's patch cells, possess antiinfectious and anticoagulant activities. The antioxidant activity of polyphenols and combinatorial antiinflammatory, anticomplementary, immunostimulating activities of polyphenols and polysaccharides have great potential in the treatment of chronic inflammatory diseases. Polysaccharide-protein complexes show antidiabetic, antiinfectious, and immunostimulating potential. Selenylated polysaccharide preparations have immune-enhancing activity and could be applied in preventive and nutritional therapy of tumor diseases.

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