CHAPTER

Clove (*Syzygium aromaticum*) polysaccharides

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1. Introduction

Clove (*Syzygium aromaticum*) belongs to the family of Myrtaceae (130–150 genera and around 3000 species); one of the most common plant families in terms of food uses and application in the food industry, thanks to their unique organoleptic and therapeutic properties (Devkota and Adhikari-Devkota, 2020). It is native to the Maluku islands in eastern Indonesia; however, recently, it has been cultivated in different regions in the world (Hussein et al., 2019). Clove tree is composed of leaves and buds, and it has a single flower of 1–2 cm long (Saeed et al., 2021). Three different stages of clove flowering bud have been reported by Kasai et al. (2016), which include phase 1 that is characterized with the green color and budding stage, phase II with the pink color and flowering stage, and the last phase with the fruiting stage and distinctive red color. They further emphasized that the clove bud's full-budding stage is the most economic value of the clove phases.

Plant-based polysaccharides are a group of bioactive natural products with a high molecular weight with increasing interest in recent years and have several attractive biological activities (Mohan et al., 2020). The approved polysaccharides for food products formulation with industrial applications are referred as food polysaccharides, with the main function of boosting the textural attributes of the foods. Naturally, these biomolecules tend to form complexes with other types of biomolecules, such as proteins and lipids (Lu et al., 2019; Yang et al., 2020; Beaumont et al., 2021). Food polysaccharides are well known for their remarkable rheological properties (thickening, stabilizing, and gelling) and for their emulsifying properties. Furthermore, their use at a very low concentration may significantly impact the textural properties of the food products (Bernaerts et al., 2019; Yang et al., 2020; Guimarães et al., 2020). Unfortunately, very little information could be found in the literature, and few studies were reported regarding the polysaccharides from clove extracts; therefore, this chapter provides an overview of the clove (*S. aromaticum*) polysaccharides composition, structure, and biological activities, as well as their reported mode/mechanism of action.

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2. Clove polysaccharides structure and composition

Carbohydrates were reported to be one of the chemical components of clove plant part extract with different concentrations based on the nature of the used plant parts and the solvent used whereby Sulieman et al. (2007) reported 51.5% content in clove bud powder. Meanwhile, Bello and Jimoh (2012) reported lower content of carbohydrates with 30.9% in clove bud, and almost the same percentage (31%) was reported by Ereifej et al. (2015). Other studies revealed carbohydrates in clove extracts on a qualitative basis only (Jimoh et al., 2017; Kaur and Kaushal, 2019).

Lee et al. (2000) compared the chemical composition of clove extracts from different regions and reported high total sugar of 26.5%–27.4% and 21.6%–21.7% for the cloves extracts from Singapore and Holland, respectively, with high arabinose (27.1% and 15.1%) and galactose (6.60% and 4.97%) for the clove extracts from Holland and Madagascar. Meanwhile, uronic acid was high (25.3%–30.3% and 25.3%–27.8%) for clove extracts from Singapore and Holland, respectively, compared to Madagascar and China clove extracts (15.2%–18.5% and 14.8%–15.2%, respectively).

In terms of structure, polysaccharides from clove belong to sulfated polysaccharides. This type of polysaccharides are extracted from natural resources or synthesized by modifying the sugar chain with sulfates (Wang et al., 2018; Lin et al., 2019; Ray et al., 2020). Two polysaccharides with relatively high antithrombotic activity were isolated from the flower buds of *S. aromaticum* (L.) Merr. & Perry (Lee et al., 2000) by anion-exchange chromatography, hydrophobic interaction column chromatography, and size exclusion chromatography with molecular weight ranging from ca. 34,000 for the low molecular weight polysaccharide (LMWPS) to the high molecular weight polysaccharide (HMWPS) of ca. 103,000 Da through the usage of butyl-toyopearl 650 M chromatography to eliminate the impurities from proteins due to the hydrophobic characteristics of resin (Lee et al., 2001).

The salt concentration (ca. 1.0 M) reported in the sulfated polysaccharide from cloves might be necessary for elution from the ion-exchange column is slightly lower than ca. 1.5 M, the concentration of algal sulfated polysaccharide (Pavão et al., 1998). This can be partly explained by the relatively low sulfate/sugar ratio in the clove-based polysaccharides. Therefore, Lee et al. (2001) assumed that the polysaccharides from cloves have distinctive structural characteristics compared to algal polysaccharides. The methylation studies on the two clove sulfated polysaccharides confirmed their polymeric complexity and heterogeneity, whereby Lee et al. (2001) reported that both clove polysaccharides are rich in pectic polysaccharides as can be deduced from the natural occurrence of variously linked residues of rhamnosyl, galactosyl, and arabinosyl (Selvendran and O'Neill, 1987). Lee et al. (2001) further added that as most of the uronic acid was shown to be galacturonic acid (GalA), very high amounts of rhamnogalacturonan were likely to be present. Remarkably, significant amounts of 4-linked Glc residues in the HMW fraction suggest the existence of appreciable cellulose in the structure.

Lee et al. (2001) detected the IR absorption bands at 818 cm^{-1} which is regarded as the C-O-S stretching vibration by sulfated polysaccharides (Perlin and Casu, 1982). The sulfates in clove polysaccharides are located at position 6 on the 3-linked Gal, and that is confirmed with the work of Nishino et al. (1994), who reported that the absorption bands at 820 cm^{-1} were caused by 6-sulfated-D-Gal.

In terms of sugar composition (molar %), LMWPS is mainly composed of 24.1% Rha, 18.9% Gal, 18.0% GalA, and 17.9% Ara with 10.8% sulfate and 18.2% protein. Meanwhile, the HMWPS consists of 26.0% Ara, 23.7% Gal, 17.5% Glc, and 12.4% Rha with 15.4% sulfate and 8.0% protein (Lee et al., 2001). Type I rhamnogalacturonan was found to be the backbone of the clove LMW and HMW polysaccharides structures with repeated units of 4-linked GalA, 2-linked Rha, and a side chain of

2,4-branched Rha with arabinan or galactan on position 4, whereby only HMW polysaccharide is likely to contain arabino-3,6-galactan moiety and this assumption is supported by the observation of a strong reactivity of the b-D-glucosyl-Yariv antigen, which reacts with arabinogalactan (Lee et al., 2001).

3. Clove polysaccharides' biological activities

Sulfated polysaccharides possess several potential biomedical applications, such as anticoagulant activity (Mourão, 2004; Fonseca et al., 2009; Zheng et al., 2021), antioxidant activity (Ahmad, 2021), antithrombotic activity (Mourão, 2004; Carvalhal et al., 2019; Chagas et al., 2020), antivirals (Andrew and Jayaraman, 2021; Hans et al., 2021), immunomodulation (Patel, 2012; Chen and Huang, 2018; Barbosa et al., 2019), and tissue engineering (Dinoro et al., 2019). For example, Lee et al. (2000) compared the anticoagulant activity of clove extracts with other herbs such as thyme, sage, oregano, and mugwort and recorded the clotting time (s) with the shortest time of ±40 s for the oregano and clove hot water extracts.

Sulfated polysaccharides from clove extracts are known to possess antithrombotic activity (Lee et al., 2001). Therefore, they presumed that the mode of action for the clove LMW polysaccharide is likely to be a low molecular weight heparin type and the heparin type for the clove HMW polysaccharide is based on the obtained results of the activated partial thromboplastin time (APTT) and thrombin time (TT) assays that are widely used to monitor the therapeutic level of heparin-induced anticoagulation (Chen et al., 2003; Eikelboom and Hirsh, 2006; Undas, 2017; McRae et al., 2021). Although polysaccharides from cloves have an antithrombotic ability, their in vitro activities are lower than that of heparin, considering that the efficacy of antithrombotic agents' comparison with heparin cannot be entirely explained in a single antithrombotic parameter (Lee et al., 2001). The antithrombotic activity of the LMW polysaccharide displayed a low activity (plasma clotting time of 90 s in APTT assay) in comparison to the HMW fraction (145 s).

The animal studies indicated that crude LMW polysaccharides did not show acute toxicity, while the acute LD_{50} of the HMW polysaccharide was approximately twofold lower than that of heparin (Lee et al., 2001). Therefore, the anticoagulation action with no toxicity in vivo of low molecular weight sulfated polysaccharide may represent a valuable and safe antithrombotic agent. The clove LMW polysaccharide has shown less in vitro antithrombotic potency than the HMW polysaccharide. It did not show the acute toxic effect in vivo while further pre-clinical studies are needed to investigate its advantages over the clove HMW polysaccharide to verify its potential application (Lee et al., 2001).

4. Conclusions

Carbohydrates form an essential component of the clove chemical structure; however, data on polysaccharides from clove are lacking, and few studies could be found in the literature. Clove polysaccharides belong to sulfated polysaccharides with molecular weight ranging from 34 to 103 kDa with promising antithrombotic activity. However, only two polysaccharides have been reported from clove extracts with approximately twofold lower acute toxicity for the HMW polysaccharide than that one reported for heparin. Therefore, further studies are recommended on clove polysaccharides to purify, characterize, and unravel their interesting structures-functions relationship using state-of-the-art analytical techniques and instrumentation to explore their potential applications.

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