POLYSACCHARIDES FROM PLANTS: A REVIEW ON METHODS OF EXTRACTION AND PURIFICATION.

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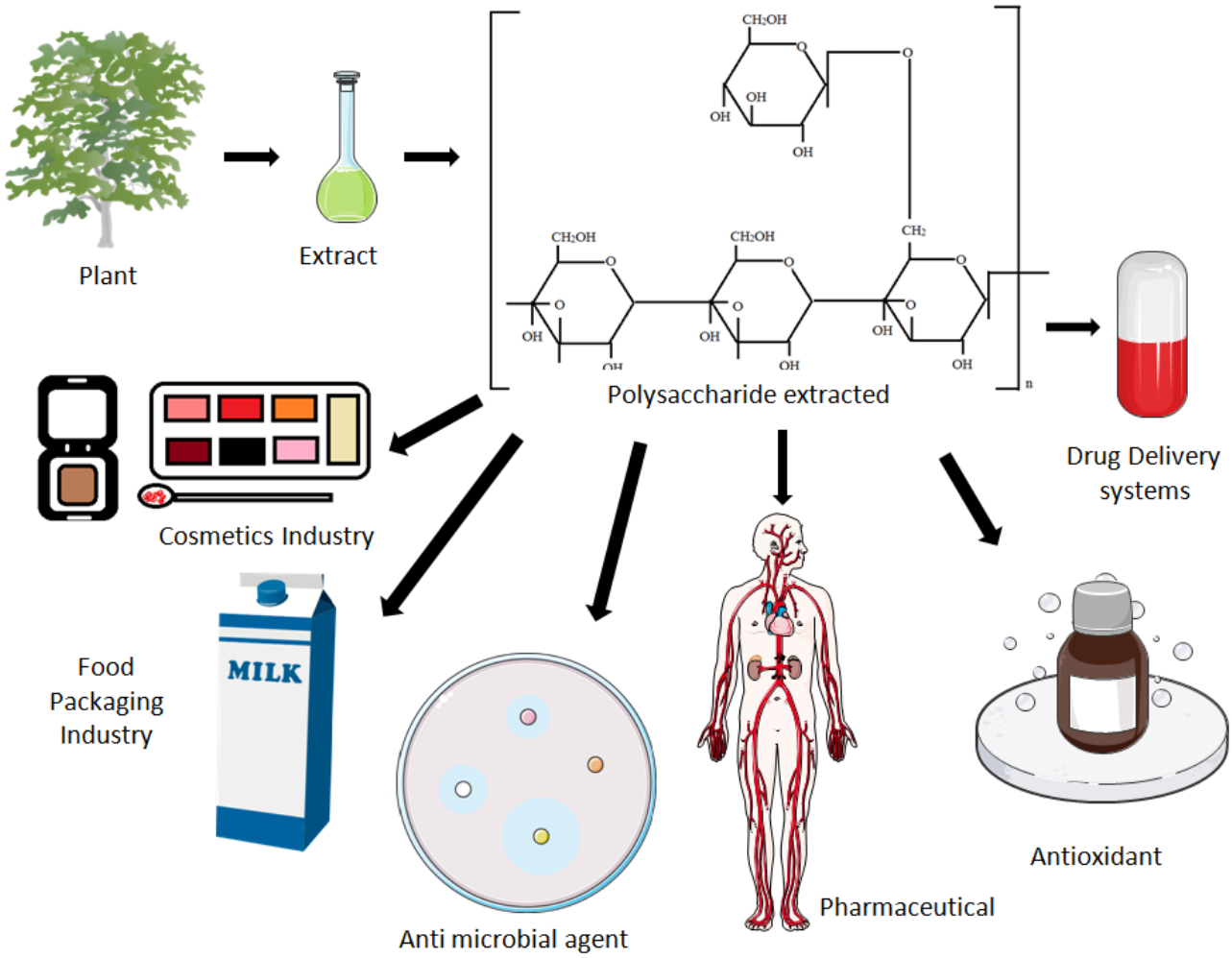
ABSTRACT:

Polysaccharides extracted from plants are of high value, because of their medicinal properties and industrial applications such as drug carriers, pharmaceutical coating, cosmetics, and food preservation coatings. Polysaccharides of different structural properties and monosaccharide composition can be extracted from plants. There are different extraction methods and purification processes, which yield polysaccharides that, varies in activity and physicochemical properties. This review paper focuses on steps related to extraction and purification of plant polysaccharides. Different methods of extraction like hot water assisted extraction, ultrasound assisted extraction, microwave-assisted extraction, enzyme assisted extraction, acid assisted extraction, alkali assisted extraction, supercritical fluid assisted extraction, aqueous two phase extraction, freeze thawing cold pressing, and liquid phase pulsed discharge are discussed in brief in this paper. The importance of optimization of extraction parameters like solvent used, temperature of extraction, power, time and yield are emphasized in this review. The important steps involved in purification of extracted polysaccharides like deproteinization methods and chromatographic techniques are also discussed.

Key words: Plants, Polysaccharides, Extraction, Purification of polysaccharides.

GRAPHICAL ABSTRACT:



1.INTRODUCTION

Polysaccharides are biological macromolecules which are polymers of monosaccharide units, may be linear or branched attributing to different properties (Yu et al., 2018). They are derived from various sources like plants, animals, bacteria, fungi, etc., (S. S. Ferreira et al., 2015). They are highly bioactive and their properties include anti-cancerous(Jia et al., 2016), antioxidant(J. Wang et al., 2016), anti-diabetic (Eseyin, 2014), anti-inflammatory (Lee et al., 2013), anti-fatigue (S. Q. Zheng et al., 2010), anti-coagulant (Q. X. Xu et al., 2016), anti-complementary, anti-viral, neuroprotective (Q. H. Gao et al., 2018), effective against inflammatory bowel disorders (C. Li et al., 2021), immunomodulatory (Xie et al., 2016), radioprotection (Jin et al.,

2013), and hepatoprotective (Zeng et al., 2016). Polysaccharides extracted from plant sources have high pharmaceutical properties. But most of the studies concentrate on flavonoids and phenolic compounds and most of the bioactivity are attributed to them. Recent studies focus on polysaccharides and their bioactivity and pharmaceutical properties.

Plant polysaccharides mainly comprises of pectin, gum, xylan, mannosan, cellulose, hemicellulose, fructosan, and starch. They play either structure based or storage based roles in plants (Caffall & Mohnen, 2009). And these polysaccharides are reported that they are conjugated to proteins, lipids and nucleotides by ionic interactions, hydrophobic interactions, steric interactions and hydrogen bonds (Dong et al., 2014). The polysaccharides also find applications in cosmetics industry, food packaging industry, medical devices industry, and drug delivery systems (Cardoso et al., 2016).

The extraction method to extract polysaccharides from plants includes hot water assisted extraction (G. tang Chen et al., 2019), ultrasound assisted extraction, microwave assisted extraction (Tao & Xu, 2008), enzyme assisted extraction (D. Y. Zhu et al., 2017), acid assisted extraction, alkali assisted extraction, chelating agent assisted extraction, hot buffer assisted extraction, accelerated solvent assisted extraction, supercritical fluid assisted extraction, aqueous two phase extraction (Z. Cheng et al., 2017), aqueous assisted extraction, cold pressing, and freeze thawing cold pressing (L. He et al., 2018), and liquid phase pulsed discharge (Boussetta et al., 2014). Polysaccharide extraction comprises of multiple steps which facilitates good solvent infiltration, dissolution, and diffusion of polysaccharides. Out of these steps solvent infiltration into the plant tissues and cells is the yield determining step in the extraction process (Caffall & Mohnen, 2009). This paper focuses on various methods of extraction of polysaccharides from plants, purification processes, and applications of polysaccharides.

2.EXTRACTION METHODS TO EXTRACT POLYSACCHARIDES:

2.1. Hot water assisted extraction

Hot water assisted extraction is one of the most used methods to extract polysaccharides from plants and natural sources. It is an eco-friendly process (X. Yang, Chen, et al., 2020) and economical with no capital expense (G. Chen, Chen, et al., 2019). It is highly preferred as polysaccharides are extracted under no complex operational conditions (Y. Huang et al., 2020). Major advantage of this process is that no harsh chemicals is used. Another advantage is that the raw materials need not be dried before the extraction process, because the water content in the raw material can be used as a solvent and as a reactant in the process (H. Zhang et al., 2020). Even though this method is less complicated and green, it requires continuous heating for long hours just to break the cell wall. The heat liberated degrades the structure of polysaccharides affecting the quality and bioactivity of the polysaccharides(G. tang Chen et al., 2019). Often this method is associated with long extraction time, high temperature and lower yields of polysaccharides. The yield of polysaccharides depends on the temperature, extraction time, number of refluxes, and solid to solvent ratio (Yun et al., 2019).

2.2. Alkali assisted extraction

The solvent used in this method is an alkaline solution. Alkalinity damages the cell wall of the plant cells which leads to the release of polysaccharides. This method is more favourable for extracting acidic polysaccharides(W. Wu et al., 2012). The important factor that affects the yield of the process is concentration of the alkaline solution(H. M. Liu et al., 2021). Higher alkaline concentration can easily break the glycosidic bonds and rupture the polysaccharide structure. This method of extraction may affect the bioactivity of the polysaccharides(Benchamas et al., 2021). Hence optimization of concentration of alkali is an important consideration to use this method.

2.3. Ultrasound assisted extraction:

Ultrasound assisted extraction method is a fast, eco-friendly, and effective technique to extract polysaccharides from plants. As ultrasonic waves propagate through the solvent, it develops cavitation enabling better penetration of solvents inside the plant cells and hence disruption of plant cells (Hammi et al., 2020). Hence the extraction efficiency is high than rest other methods(Sorourian et al., 2020). As ultrasonic waves are used to lyse the cells and extract polysaccharides, there is no need of high temperature and long-time(Ebringerová & Hromádková, 2010). Thus there is minimal damage to the structural properties and molecular properties of the polysaccharide extracted. It has gained more attention from researchers due to the higher extraction efficiency(X. Guo et al., 2017). The main disadvantage of this method is that the ultrasound may destroy the polysaccharide structure. So care should be taken about the frequency of waves used (Y. Wang et al., 2019). This method can be easily combined with other extraction methods like enzyme assisted extraction, subcritical fluid extraction, etc., to improve the yield of polysaccharides (X. X. Liu et al., 2020).

2.4. Microwave assisted extraction:

Microwave assisted extraction process is an efficient technique used to extract bioactive components from various biological sources. The microwaves are non-ionizing radiation with a frequency of 0.3~300 GHz and with high penetrability(D. Xu et al., 2020)and can produce volumetrically distributed heating inside the plant cells and tissues through dipolar and ionic interactions between the electromagnetic field and bioactive components(Z. Hu et al., 2018). They penetrate the plant tissues and reach the inner glandular and vascular systems causing sudden rise in the temperature. This heat causes vaporization of volatile components and rise in the intracellular pressure. This rise in pressure makes the cells explode and the active

components are released (Rostami & Gharibzahedi, 2016). Diffusion coefficient and mass transfer of the polysaccharides can be increased by using the dipolar interaction with polar solvents and the conductive migration of dissolved ions (W. Wang & Liu, 2020). However the stability and bioactivity of the polysaccharides is significantly affected by microwave power. So it is important to optimize the conditions of extraction. Because of the advantages like lower extraction time, good quality end product, high yield, less laborious and less consumption of solvent (Rostami & Gharibzahedi, 2017). It is also eco-friendly and only requires less solvent and energy (Delazar et al., 2012). The properties that affect the extraction yield are microwave power, extraction time, solid to liquid ratio and temperature.

2.5. Enzyme assisted extraction:

Enzyme assisted extraction is mild, eco-friendly, energy saving and efficient method to extract polysaccharides from plant materials. The cell wall of plant cells is mainly composed of cellulose, hemicellulose, lignin, pectin, protein and some inorganic compounds(Y. Guo et al., 2020). Enzymes like cellulase, protease, pectinase, and α –amylase can catalyse the degradation of plant cell wall and promotes the release of polysaccharide from plant materials (Song et al., 2020).Use of enzyme offers specificity and selectivity, as enzymes specifically degrades the cell wall structures(Y. Li, Qin, et al., 2020). The cell wall and membranes can be rapidly disrupted and polysaccharides can be released rapidly(X. Chen, Zhang, et al., 2020). Using this method higher yield of polysaccharides with minimal impurities can be obtained. Enzymatic extraction improves the extraction efficiency as the enzyme reaction is specific only to the cell wall material of plants. Due to this specificity the biological activity of the polysaccharides are restored(Jia et al., 2016). But the effectiveness mainly depends on the plant material. Also enzymatic extraction can be used to extract polysaccharides that are different in properties from the same material. Even though this method is very advantageous, it is not preferred in large scale due to the cost associated with the use of enzymes (Rostami & Gharibzahedi, 2017). Hence

there is a need to optimize the enzyme dosage and time of extraction for better yield within the constraints of economics.

2.6. Subcritical fluid assisted extraction (SFE):

Subcritical water extraction is one of the recently used method to extract polysaccharides from plants (Chao et al., 2013; X. Luo et al., 2017). Hot water is maintained under pressure of about 1 – 22.1 MPa to keep the water in liquid phase as solvent (Z. Y. Ju & Howard, 2005; Ramos et al., 2002; Soto Ayala & Luque de Castro, 2001). It is preferred because of its high yield, low cost, eco-friendly and convenience (X. Luo et al., 2017). The principle of this method is to separate molecules by using the effects of pressure and temperature on the solubility and diffusion of the solvent used(Jia et al., 2016). This type of extraction is carried out in very low temperature. Polysaccharides can be extracted without any solvent residue and in higher quantity(S. Zhang et al., 2021). SCF extraction (Subcritical Fluid) is green without any pollution, but this method requires a specialized instrumentation facility of high cost which limits its application(Mena-García et al., 2019) and it has almost no effect on the properties of the polysaccharides (Chao et al., 2013; X. X. Liu et al., 2020).

2.7. Ultra-High-Pressure assisted Extraction:

The principle of this method is to rupture cell wall of plant cells under very high pressure. Higher pressure keeps the solvent in liquid state higher than its boiling point, increasing the extraction kinetics(S. Zhang et al., 2021). Some of the advantages of this method are high efficiency, simple operation, less solvent consumption, short extraction time, and less substrate influence. As the cell wall gets ruptured rapidly, various intracellular impurities get co extracted. This is an energy consuming process.

2.8. Dynamic high-pressure micro fluidization (DHPM):

Dynamic High Pressure micro fluidization is an emerging technology which utilizes collective forces of high shear, cavitation, high frequency vibration, instantaneous pressure drop and rise in pressure up to 200 MPa (W. Liu et al., 2010).This methodology utilizes low pressure to extract polysaccharides from plant cells (X. Huang et al., 2012). The cells collide with each other rapidly and burst due to instant low pressure. This technique can be characterized by short extraction time, high extraction yield, mild extraction condition and good quality product. DHPM causes the cellular contents to overflow due to the constant change in pressure and improves the yield of polysaccharides (L. Zhang, Tu, et al., 2015). Energy produced from DHPM was mainly used to modify biological macromolecules like proteins (W. Liu et al., 2010), fibres (Tu et al., 2012), polysaccharides (C. Liu et al., 2016) like starch (N. Wang et al., 2021), and enzymes (W. Liu et al., 2010) extracted from plants. DHPM is a potential homogenization technique with non-thermal processing, that can change the rheological behaviours like viscosity, gel properties and viscosity (X. Huang et al., 2012; W. Liu et al., 2010; Tu et al., 2012).

2.9 Freeze thawing cold pressing Extraction:

Freeze thawing cold pressing is a new innovative method to extract polysaccharides by breaking the cell wall by freezing and immediate thawing and causing the polysaccharides to diffuse out. A report on comparative study while using different methods like conventional hot water extraction, cold-pressing, freeze-thawing cold-pressing, ultrasonic-assisted hot water extraction, microwave-assisted hot water extraction and enzyme-assisted hot water extraction for extracting polysaccharides from the stem of *Dendrobium officinale* reported that polysaccharide extracted using freeze-thawing cold-pressing showed high extraction yield, well-preserved molecular chains and best antioxidant activity. Additionally this method prevents degradation of polysaccharides by heat and ultrasound (L. He et al., 2018). In this method after homogenization

of fresh stem, homogenate was placed in -80°C for 24 h, and then quickly thawed at 60°C. This resulted in better release of the polysaccharides into solution. The polysaccharides were precipitated from the resulting solution using ethanol post removal of stem residues.

2.10 Liquid phase pulsed discharge (LPD) assisted Extraction:

Liquid phase pulsed discharge is a novel non thermal technique which utilises electric discharge in water causing both physical phenomena and chemical reactions. It is comparatively an efficient method(J. Li et al., 2020). The amplitude shocks, liquid turbulence, and cavitation facilitates the breakage of cells and tissues thereby causing the diffusion of polysaccharides (Boussetta et al., 2014). In this technique electric pulses are passed through the liquid phase i.e., the solvent phase. The electric pulses create pores in the cell membrane of the plant cells and accelerates the release of polysaccharides. This technique was developed on the basis that electrical breakdown in water causes both chemical and physical reactions(T. Ju & Xi, 2020). The chemical reactions like UV light emission and free radical formation and physical phenomena like cavitation and high amplitude shock waves cause the mass transfer to increase. This leads to the breakage of cell wall and facilitates the release of polysaccharides inside the cells. This method has proved to have many advantages like high yield with short extraction time and low temperature rise (only up to 5°C) and is usually used to extract oil based functional compounds like tocopherols, phytosterols and polyphenols (T. Ju et al., 2019). LPD instrumentation consists of a LPD pretreatment unit and a solid liquid extraction device. The plant samples and a little quantity of solvent is treated first and then extract is carried out with large quantity of solvent. The factors that affect the extraction efficiency are frequency of electric pulses, electric field strength and particle size of the plant material(J. Li et al., 2020). Some of the disadvantages of this method are laborious operating procedures, cannot be operated in continuous mode, and difficult to scale up for large scale industrial production (Boussetta et al., 2014).

2.11 Extraction using Deep Eutectic Solvents:

Current studies show the emergence of green solvents, which are eco-friendly and equivalents to organic solvents. They are named Deep Eutectic Solvents (DES) (Z. Li et al., 2018). They are synthesized by mixing a hydrogen bond donor and a hydrogen bond acceptor. They are said to be excellent solvents with useful properties like nontoxic, non-flammable and biodegradable(X. Shang et al., 2019). And due to all these properties they have found their application in biochemistry, nanotechnology, biocatalysis and extraction of bioactive molecules from plants (Cao et al., 2018). Especially, the combination of DES and microwave extraction is reported to be highly efficient (Nie et al., 2017).

2.12 Methods to extract polysaccharides from plants:

The various methods of polysaccharides extraction from different plants are listed in the table 2.1. The extraction process utilizes aqueous solvents with variations in pH, temperature, and solid solvent ratio. The conventional method used in the extraction of polysaccharides from biological samples utilized hot water which was time consuming and generally required high temperature. But alternative methodologies like ultrasound assisted extraction, enzyme assisted extraction and microwave assisted extraction are operated at short times to provide higher yields. All of these methods have their own merits and demerits. Statistical optimisation of extraction parameters would enable to identify the optimized conditions for obtaining higher yield within shorter time and greater structural integrity and biological activity of extracted polysaccharides.

Plant	Extraction method	Solid to liquid ratio (g:ml)	Solvent	Time	Temperature	Yield %	Reference
Allium cepa	Hot buffer assisted extraction	1:30	Sodium acetate buffer (0.05 M, pH5.2)	1 h	70 ℃	83.92 ± 2.08 (mg/g Alcohol insoluble solids)	(D. Y. Zhu et al., 2017)
	Chelating agent assisted extraction	1:30	0.05 M ammonium oxalate and 0.05 M EDTA-2Na in sodium acetate buffer (0.05 M, pH5.2)	1 h	70 ℃	34.50 ± 1.64 (mg/g Alcohol insoluble	

Plant	Extraction method	Solid to liquid ratio (g:ml)	Solvent	Time	Temperature	Yield %	Reference
						solids)	
	Diluted alkalineassisted extraction	1:30	0.05 M sodium hydroxide and 20 mm Sodium borohydride	1 h	4 °C	52.65 ± 2.56 (mg/g Alcohol insoluble solids)	
	Concentrated alkaline assisted extraction	1:30	6 M sodium hydroxide and 20 mm Sodium borohydride	2 h	4 °C	129.82 ± 1.08 (mg/g	

Plant	**Extraction method**	**Solid to liquid ratio (g:ml)**	**Solvent**	**Time**	**Temperature**	**Yield %**	**Reference**
						Alcohol insoluble solids)	
Allium sativum	Hot water assisted extraction	1:10	Distilled water	2.5 h	80 °C	-	(H. Cheng & Huang, 2018)
Alpiniaeoxyphyllae	Hot water assisted extraction	1:12	Distilled water	1 h	90 °C	3.18	(X. Yang, Yang, et al., 2020)
Arctium lappa L	Ultrasound assisted	1:31	Distilled water	83 min at 158 W	50 °C	8.22	(Y. yuan Jiang et al.,

Plant	Extraction method	Solid to liquid ratio (g:ml)	Solvent	Time	Temperature	Yield %	Reference
	extraction			power			2019)
Artemisia selengensis	Ultrasound assisted extraction	-	Distilled water	14.5 min at 146 W power	60 °C	8.86	(J. Wang et al., 2016)
Bellamya quadrata	Enzyme assisted extraction	1:24	Protease solution (285 U/g) pH4.71	1 h	67 °C	9.87	(Xiong et al., 2020)
Broussonetia papyrifera	Ultrasound assisted extraction	1:30	Distilled water	50 min extraction power 180W	60 °C	8.61	(Han et al., 2016)

Plant	Extraction method	Solid to liquid ratio (g:ml)	Solvent	Time	Temperature	Yield %	Reference
Camellia oleifera seed cake	Ultrasound assisted extraction	1:30	Deep eutectic solvent (Choline Chloride - Ethylene Glycol in the ratio 1:2)	50 min	50 °C	12.19	(C. Gao et al., 2020)
Camptotheca acuminata	Microwave assisted extraction	1:40	Distilled water	14 min 600 W power	70 °C	8.61	(W. Hu et al., 2019)
Carex meyeriana	Hot water assisted extraction	1:29.25	Distilled water	1.66 h	95 °C	0.47	(Z. Hu et al., 2018)
Chimonobambusa quadrangularis	Accelerated solvent assisted	5 g plant powder	Distilled water	22 min with static cycles	126 °C	9.9	(G. Chen, Fang, et al.,

Plant	Extraction method	Solid to liquid ratio (g:ml)	Solvent	Time	Temperature	Yield %	Reference
	extraction			2 in Buchi speed extractor E-914 system			2019)
	Hot water assisted extraction	1:20	Distilled water	4 h	100 °C	7.2	
	Ultrasound assisted extraction	1:20	Distilled water	40 min 240 W ultrasonic power	49 °C	8.7	

Plant	Extraction method	Solid to liquid ratio (g:ml)	Solvent	Time	Temperature	Yield %	Reference
	Microwave assisted extraction	1:20	Distilled water	15 min 400 W microwave power	90 ℃	8.2	
	Enzyme assisted extraction	1:20	1% (w/v) Enzyme solution in distilled water (Cellulase, papain, pectinase in 1:1:1)	80 min	50 ℃	8.3	
Chinese dates	Hot water assisted extraction	1:20	Distilled water	160 min	80 °c	7.2	(Y. Zhao et al., 2014)

Plant	Extraction method	Solid to liquid ratio (g:ml)	Solvent	Time	Temperature	Yield %	Reference
	Alkali assisted extraction	1:10	First with distilled water at 60 °C. The residue was then treated with 0.1 N of sodium hydroxide solution	1 hr	30 °C	3.3	(X. Lin et al., 2019)
	Ultrasound assisted extraction	1:26.3	Distilled water	21.2 min 134.9 W ultrasonic power	52.5 °C	1.05	(T. Lin et al., 2018)
	Microwave assisted extraction	1:30	Distilled water	60 min 400 W microwave power	75 °C	9.02	(Rostami & Gharibzahedi, 2016)

Plant	Extraction method	Solid to liquid ratio (g:ml)	Solvent	Time	Temperature	Yield %	Reference
	Subcritical water assisted extraction	1:20	Subcritical water	60 min	140 ℃	7.9	(X. X. Liu et al., 2020)
Coffee grounds	Microwave assisted extraction	1:10	Distilled water	10 min	170 ℃	21.5 (Soluble Sugars)	(Passos et al., 2019)
Symphytum officinale L	Hot water assisted extraction	1:25	Distilled water	155 min	89 ℃	19.2	(H. Shang et al., 2020)
Crataegus	Hot water assisted	1:20	Distilled water	2 h	90 ℃	5.88	(X. Chen, Zhang, et al.,

Plant	Extraction method	Solid to liquid ratio (g:ml)	Solvent	Time	Temperature	Yield %	Reference
pinnatifida	extraction						2020)
	Ultrasound assisted extraction	1:20	Distilled water	60 min	60 ℃	7.47	
	Enzyme assisted extraction	1:30	5% (w/v) solution of Cellulase	90 min	55 ℃	9.59	
	Enzyme-ultrasound assisted extraction	1:30	5% (w/v) solution of Cellulase	66 min	52 ℃	10.39	

Plant	**Extraction method**	**Solid to liquid ratio (g:ml)**	**Solvent**	**Time**	**Temperature**	**Yield %**	**Reference**
Curcubita moschata	Enzyme assisted extraction	-	4000 U/g solution of Cellulase	80 min	55 °C	15.4	(Umavathi et al., 2021)
	Hot water assisted extraction	1:5	Distilled water	10 h	80 °C	-	(L. Chen et al., 2020)
Cucurbita argyrosperma	Hot water assisted extraction	1:10	Distilled water	30 min	100 °C	-	(F. Chen & Huang, 2018)
				2 h	60 °C		
Cyphomandra betacea	Microwave assisted extraction	1:40	Distilled water	2 h 400 W microwave power	60 °C	36.52	(C. et al., 2016)

Plant	Extraction method	Solid to liquid ratio (g:ml)	Solvent	Time	Temperature	Yield %	Reference
Taraxacum officinale	Hot water assisted extraction	1:25	Distilled water	4 h	100 °C	44.95	(L. Cai et al., 2019)
Dendrobium officinale	Cold pressing extraction	1:20	Distilled water added and extract obtained with the help of juicer	-	-	13.779	(L. He et al., 2018)
	Freeze thawing Cold pressing Extraction	1:20	Homogenised with Distilled water	24 h	-80 °C	20.33	
	Hot water assisted extraction	1:20	Homogenised with Distilled water	3 h	100 °C	14.77	

Plant	Extraction method	Solid to liquid ratio (g:ml)	Solvent	Time	Temperature	Yield %	Reference
	Ultrasound assisted extraction	1:20	Distilled water	2 h	65 ℃	20.55	
	Microwave assisted extraction	1:20	Distilled water	90 s	-	17.74	
	Enzyme assisted extraction	1:20	0.15% (w/v) Cellulase solution pH-5	3 h	55 ℃	18.5	
Fragaria vesca	Cold Alkali assisted extraction	1:10	0.1 N Sodium hydroxide	24 h	Room temperature	2.5	(Pawlaczyk-Graja et al., 2019)

Plant	Extraction method	Solid to liquid ratio (g:ml)	Solvent	Time	Temperature	Yield %	Reference
	Hot alkali assisted extraction	1:10	0.1 N Sodium hydroxide	6 h	100 ℃	7.7	
	Ultrasound assisted extraction	1:10	Distilled water	40 min 60 W	25 ℃	4.9	
	Microwave assisted extraction	1:10	Distilled water	20 min 200 W	80 ℃	6.7	
Fritillaria pallidiflora	Ultrasound assisted	1:20	Distilled water	50 min 500 W	50 ℃	20.77	(Abuduwaili et al., 2019)

Plant	Extraction method	Solid to liquid ratio (g:ml)	Solvent	Time	Temperature	Yield %	Reference
	extraction						
	Enzyme assisted Extraction	1:20	0.5% (w/w) Cellulase solution pH 5.5	6 h	50 ℃	9.89	
	Enzyme-ultrasound assisted extraction	1:20	0.5% (w/w) Cellulase solution pH 5.5	50 min 500 W	50 ℃	23.27	
			0.5% (w/w) Pectinase solution pH 5.5	50 min 500 W	50 ℃	10.56	
Fructus meliae toosendan	Microwave assisted extraction	1:30000	Distilled water	20 min 700 W	60 ℃	15.75	(L. Xu et al., 2018)

Plant	Extraction method	Solid to liquid ratio (g:ml)	Solvent	Time	Temperature	Yield %	Reference
Morus alba	Hot water assisted extraction	1:40	Distilled water	2 h	90 °C	1.58	(C. Chen et al., 2019)
	Acid Assisted extraction	1:40	0.1 N Hydrochloric acid	2 h	90 °C	1.93	
	Alkali assisted extraction	1:40	0.1 N Sodium hydroxide	2 h	90 °C	2.25	
	Ultrasound assisted extraction	1:53	Distilled water	80 min	57 °C	6.92	(D. Y. Zhang et al., 2016)
		1:40	Distilled water	2 h	90 °C	2.97	(C. Chen et

Plant	Extraction method	Solid to liquid ratio (g:ml)	Solvent	Time	Temperature	Yield %	Reference
							al., 2019)
Ganoderma lucidum	Green extraction	1:30	50% Deep eutectic solvent aqueous solution (Ethanolamine - o-cresol in the ratio 1:1)	50 min	60 ℃	9.235	(C. Cai et al., 2020)
Gentiana scabra	Microwave assisted aqueous two-phase extraction	1:21	Ethanol mass fraction 21.73% and Sodium dihydrogen phosphate mass fraction 23.27% for the ATPS	5.8 min 800 W	95 ℃	15.97% in top phase and 16.55% in bottom	(Z. Cheng et al., 2017)

Plant	Extraction method	Solid to liquid ratio (g:ml)	Solvent	Time	Temperature	Yield %	Reference
						phase	
Zingiber officinale	Hot water assisted extraction	1:40	Distilled water	120 min	70 ℃	12.13	(G. tang Chen et al., 2019)
	Ultrasound assisted extraction	1:40	Distilled water	17 min 400 W	74 ℃	16.62	
Ginkgo biloba seed	Hot water assisted	1:10	Distilled water	3 h	70-80 ℃	4.87	Li. X. H., Yang. Q., &

Plant	Extraction method	Solid to liquid ratio (g:ml)	Solvent	Time	Temperature	Yield %	Reference
	extraction						Wang. L., 2012
Ginkgo biloba leaves	Hot water assisted extraction	1:30	Distilled water	3.5	80 ℃	9.025	(B. Jiang et al., 2010)
	Ultrasound – enzyme assisted extraction	1:30	PEG - Enzyme complex (Cellulase: pectinase: trypsin in the ratio 2:2:1) pH- 4.34	37.13 min	51.88 ℃	7.29	(L. Zhang, Guo, et al., 2015)

Plant	**Extraction method**	**Solid to liquid ratio (g:ml)**	**Solvent**	**Time**	**Temperature**	**Yield %**	**Reference**
Grifola fondosa	Super critical fluid assisted extraction	Supercritical CO_2 at a flowrate of 100 L/h and 2.86 ml/g entrainer	Supercritical Carbondioxide	116.3 min at 34.5 MPa	36.7 ℃	4.61	(H. K. Zhao et al., 2021)
Psidium guajava leaves	Ultrasound assisted extraction	1:10	Distilled water	20 min 404 W	62 ℃	1	(Y. Luo et al., 2018)
Hippophae rhaminodes pomace	Microwave assisted	1:10	Distilled water	6 min 600 W	85 ℃	0.264	(Wei et al., 2019)

Plant	Extraction method	Solid to liquid ratio (g:ml)	Solvent	Time	Temperature	Yield %	Reference
	extraction						
Hovenia dulcis	Multifrequency Ultrasound assisted extraction	1:20	Distilled water	33 min 28 and 40 khz	58 ℃	9.02	(B. Yang, Luo, et al., 2020)
Syzygium cumini seeds	Microwave assisted extraction	1:15	Distilled waterpH3.2	3.1 min 515 W	-	4.71	(Al-Dhabi & Ponmurugan, 2020)
Lentinus edodes	Enzyme assisted extraction	1:29	15, 20, 15 g/kg of plant powder cellulase, papain and pectinase	93 min	54 ℃	15.65	(Y. ming Zhao et al.,

Plant	Extraction method	Solid to liquid ratio (g:ml)	Solvent	Time	Temperature	Yield %	Reference
			solution pH5				2016)
Lillium lancifolium	Hot water assisted extraction	1:25	Distilled water	4 h	90 ℃	22.77	(J. hua Huang et al., 2020)
Lillium brownii						27.3	
Lilliumda vidiidar						24.6	
Nelumba nucifera leaves	Hot water assisted extraction	1:20	Distilled water	4 h	100 ℃	1.18	(Song et al., 2020)
	Enzyme assisted extraction	1:20	1% v/w of raw material amylase solution pH5	48 h	50 ℃	0.97	

Plant	Extraction method	Solid to liquid ratio (g:ml)	Solvent	Time	Temperature	Yield %	Reference
			1% v/w of raw material cellulase solution pH5			1.17	
			1% v/w of raw material pectinase solution pH5			1.11	
			1% v/w of raw material protease solution pH7			1.93	
Luffa cylindrica	Ultrasound assisted extraction	1:50	Distilled water	90 min	75.8 ℃	6.56	(Y. Wang et al., 2019)
Magnolia	Hot water assisted	1:20	Distilled water	4 h	Heated under reflux	5.09	(Y. F. Zheng

Plant	Extraction method	Solid to liquid ratio (g:ml)	Solvent	Time	Temperature	Yield %	Reference
kwangsiensis	extraction				conditions		et al., 2016)
Mangifera indica	Ultrasound assisted extraction	1:40	Distilled water	100 min 170 W	74 ℃	3.89	(H. Hu et al., 2018)
Althaea officinalis	Microwave assisted extraction	1:40	Distilled water	26 min 457.32 W	75 ℃	14.47	(Hashemifesh araki et al., 2020)
Mentha haplocalyx	Hot water assisted extraction	1:20	Distilled water	3 h	95 ℃	6.21	(Fang et al., 2020)

Plant	Extraction method	Solid to liquid ratio (g:ml)	Solvent	Time	Temperature	Yield %	Reference
	Acid assisted extraction		Citric acid solution pH3			7.28	
	Alkali assisted extraction		5% sodium hydroxide/ 0.05% sodium borohydride solution			9.37	
	Saline assisted extraction		0.9% sodium chloride solution			7.78	
Momordica charantia	Hot water assisted extraction	1:10	Distilled water	3 h	50 ℃	-	(X. Yang, Chen, et al., 2020)
Morinda citrifolia	Hot water assisted	1:41.9	Distilled water	117.6 min	77.7 ℃	9.19	(J. Li et al.,

Plant	Extraction method	Solid to liquid ratio (g:ml)	Solvent	Time	Temperature	Yield %	Reference
	extraction						2020)
	Ultrasound assisted extraction	1:33.3	Distilled water	81.7 min	78 ℃	11.13	
	Pulse electric field assisted extraction	1:30	Distilled water	6.1 kV/cm electric field strength for 77 times and then for 82.14 min	76.4 ℃	10.8	

Plant	Extraction method	Solid to liquid ratio (g:ml)	Solvent	Time	Temperature	Yield %	Reference
Notopterygium franchetii	Microwave assisted Aqueous two phase extraction	1:40	Ethanol mass fraction 32% and Sodium dihydrogen pHospHate mass fraction 24% for the ATPS	15 min	30 ℃	80.57	(W. Wang & Liu, 2020)
Abelmoschus esculentus	Aqueous assisted extraction	1:150	Distilled water	30 min	20-30 ℃	29.4	(Y. Li, Wang, et al., 2020)
	Hot water assisted extraction	1:40	Distilled water	1 h	70 ℃	7.68 - 20.15	(Bai et al., 2020)
Operculina macrocarpa	Hot water assisted	1:50	Distilled water	30 min	85 ℃	6.41 - 8.07	(Galvão et al., 2014)

Plant	Extraction method	Solid to liquid ratio (g:ml)	Solvent	Time	Temperature	Yield %	Reference
	extraction						
Orchis chusua	Ultrasound assisted extraction	1:40	Distilled water	50 min 390 W	60 ℃	48.3	(Nuerxiati et al., 2019)
Ornithogallum billardieri	Ultrasound assisted extraction	1:35.3	Distilled water	38.5 min	43.5 ℃	85.9	(Medlej et al., 2020)
Paeoniae radix alba	Hot water assisted extraction	1:10.65	Distilled water	2.1 h repeated 2 times	Heated under reflux conditions	10.17	(S. Wang et al., 2020)

Plant	**Extraction method**	**Solid to liquid ratio (g:ml)**	**Solvent**	**Time**	**Temperature**	**Yield %**	**Reference**
Oryza sativa	Hot water assisted extraction	1:15	Distilled water	2 h repeated twice	90 ℃	0.8	(Surin et al., 2020)
	Ultrasound assisted extraction	1:20	Distilled water	20 min	70 ℃	4	
Arachis hypogaea Oil cake sediments	Aqueous assisted extraction	1:20	Distilled water pH-4	40 min	121 ℃	39.6	(Ye et al., 2019)
Punica granatum peels	Enzyme assisted extraction	1:22	0.93% Cellulase solution pH-5	88 min	55 ℃	22.35	(Y. Li et al., 2018)

Plant	Extraction method	Solid to liquid ratio (g:ml)	Solvent	Time	Temperature	Yield %	Reference
Poria cocos	Green extraction	1:20	Deep Eutectic Solvent solution choline chloride: oxalic acid in the ratio 1:2	15 min	100 ℃	46.24	(W. Zhang et al., 2020)
	Hot water assisted extraction	1:20	Distilled water	1 h	100 ℃	5.4	
Potentilla anserina	Hot water assisted extraction	1:22	Distilled water	4 h	81 ℃	8.97	(Y. Huang et al., 2020).
	Ultrasound assisted	1:22	Distilled water	2.6 h 205 W	66 ℃	9.43	

Plant	Extraction method	Solid to liquid ratio (g:ml)	Solvent	Time	Temperature	Yield %	Reference
	extraction						
Pouteria campechiana seeds	Ultrasound assisted extraction	1:41	Distilled water	69 min	79 ℃	15.94	(Ma et al., 2020)
Hordium vulgare	Hot water assisted extraction	1:20	Distilled water	2 h	50 ℃	2.93	(J. L. He et al., 2020)
	Ultrasound assisted extraction	1:40	Distilled water	20 min 70% amplitude	Room temperature	3.28	

Plant	Extraction method	Solid to liquid ratio (g:ml)	Solvent	Time	Temperature	Yield %	Reference
	Pressurized water extraction	1:30	Distilled water	40 min 1.6 MPa	55 ℃	3.56	
	Microwave assisted extraction	1:30	Distilled water	10 min 480 W	85 ℃	3.62	
Rhododendron aganniphum	Ultrasound assisted extraction	1:25	Distilled water	2.2 h 200 W	55 ℃	9.428	(X. Guo et al., 2017)
	Hot water assisted extraction	1:25	Distilled water	12 h	55 ℃	8.451	

Plant	Extraction method	Solid to liquid ratio (g:ml)	Solvent	Time	Temperature	Yield %	Reference
Ribes nigrum	Microwave assisted extraction	1:31	Distilled water	41 min 414 W	30 ℃	10.59	(Y. Yang, Lei, et al., 2020)
	Hot water assisted extraction	1:30	Distilled water	2 h	80 ℃	5.3	
Oryza sativa bran	Three phase partitioning system assisted extraction	1:20	Ammonium sulphate concentration 28% (w/v), slurry to t -butanol ratio 1:1.1 (v/v), pH 5.1	1 h	40 ℃	2.09	(Surin et al., 2020)

Plant	Extraction method	Solid to liquid ratio (g:ml)	Solvent	Time	Temperature	Yield %	Reference
Rosa roxburghii leaves	Hot water assisted extraction	1:21.16	Distilled water	90.49 min	81.32 ℃	11.04	(H. Wu, Li, et al., 2020)
Salvia miltiorrhiza	Hot water assisted extraction	1:20	Distilled water	4 h	70 ℃	-	(W. Wu et al., 2012)
	Ultrasound assisted extraction	1:20	Distilled water	40 min	Room temperature	-	
	Alkali assisted extraction	1:20	0.5N Sodium hydroxide	40 min	Room temperature	-	

Plant	Extraction method	Solid to liquid ratio (g:ml)	Solvent	Time	Temperature	Yield %	Reference
	Enzyme assisted extraction	1:10	Distilled water + 5g cellulase	2 h	60 ℃	-	
Silphium perfoliatum	Enzyme assisted extraction	1:22	1.59% Enzyme complex dosage (pectinase, papain, cellulase) pH5.1	66 min	50 ℃	13.69	(Y. Guo et al., 2020)
		1:15	1% Enzyme complex dosage (pectinase, papain, cellulase) pH5.1	61 min	55 ℃	9.87	(H. Wu, Shang, et al., 2020)
	Hot water assisted extraction	1:15	Distilled water	61 min	97 ℃	6.44	
	Ultrasound assisted	1:15	Distilled water	61 min 100	55 ℃	8.53	

Plant	Extraction method	Solid to liquid ratio (g:ml)	Solvent	Time	Temperature	Yield %	Reference
	extraction			W			
	Enzyme - ultrasound assisted extraction	1:15	1% Enzyme complex dosage (pectinase, papain, cellulase) pH- 5.1	61 min 100 W	55 ℃	9.31	
Sinonovascula constricta	Enzyme assisted extraction	1:20	4% Enzyme complex dosage (pectinase, papain, cellulase) pH- 8.2	173 min	50 ℃	17.72	(Yuan et al., 2020)
	Hot water assisted	1:30	Distilled water	4 h	80 ℃	5.46	

Plant	**Extraction method**	**Solid to liquid ratio (g:ml)**	**Solvent**	**Time**	**Temperature**	**Yield %**	**Reference**
	extraction						
Helianthus annuus oil meal	Alkali assisted extraction	1:20	2 M NaOH solution	5 h refluxed three times	50 ℃	8	(H. M. Liu et al., 2021)
Zanthoxylum armatum seeds	Microwave assisted extraction	1:44	Distilled water	16 min 500 W	80 ℃	4.76	(D. Xu et al., 2020)
Trichosanthes kirilowii	Microwave assisted extraction	1:42	Distilled water	26 min 570 W	80 ℃	2.43	(Z. Hu et al., 2020)

Plant	Extraction method	Solid to liquid ratio (g:ml)	Solvent	Time	Temperature	Yield %	Reference
Trifolium pratense	Hot water assisted extraction	1:21	Distilled water	95 min	93 ℃	12.72	(H. Zhang et al., 2020)
Trifolium repens	Hot water assisted extraction	1:20	Distilled water	90 min	90 ℃	8.35	(H. Shang et al., 2019)
	Ultrasound assisted extraction	1:20	Distilled water	90 min 100 W	55 ℃	9.43	
	Enzyme assisted extraction	1:20	1% Enzyme complex dosage (cellulase, papain and pectinase)	90 min	55 ℃	10.57	

Plant	**Extraction method**	**Solid to liquid ratio (g:ml)**	**Solvent**	**Time**	**Temperature**	**Yield %**	**Reference**
	Ultrasound - Enzyme assisted extraction	1:20	First - Distilled water	45 min 100 W	55 ℃	10.62	
			Second - 1% Enzyme complex dosage (cellulase, papain and pectinase)	45 min			
Tuber aestivum	Ultrasound assisted extraction	1:75	Distilled waterpH6.5	15 min	Room temperature	68.91	(Mudliyar et al., 2019)
	Hot water assisted	1:20	Distilled water	10 h	90 ℃	28.36	

Plant	Extraction method	Solid to liquid ratio (g:ml)	Solvent	Time	Temperature	Yield %	Reference
	extraction						
Zizhyphus lotus	Hot water assisted extraction	1:39	Distilled water	3 h 15 min	91.2 ℃	18.88	(Hammi et al., 2020)
Curcuma longa	Hot water assisted extraction	1:20	Distilled water	2.5 h	100 ℃	2.23	(Z. Zhu et al., 2022)
Typha domingensis	Ultrasonic assisted alkali extraction	1:25	1.5 M naohsolution	40 min	70 ℃	12.24	(Sorourian et al., 2020)

Plant	Extraction method	Solid to liquid ratio (g:ml)	Solvent	Time	Temperature	Yield %	Reference
Wheat germ	Hot water assisted extraction	1:5	Distilled water	44 min three times	69 ℃	8.89	(Yun et al., 2019)
Ziziphus jujube	Ultrasound assisted extraction	1:33.5	Distilled water	100 min 140 W	83.1 ℃	1.97	(Z. Wu et al., 2019)
Zingiber officinale (stem and leaves)	Hot water assisted extraction	1:20	Distilled water	5 h	100 ℃	5.5	(X. Chen, Chen, et al., 2020)
	Ultrasound assisted	1:20	Distilled water	60 min 300 W	50 ℃	7.17	

Plant	Extraction method	Solid to liquid ratio (g:ml)	Solvent	Time	Temperature	Yield %	Reference
	extraction						
	Alkali assisted extraction	1:20	0.2 M alkaline solution	2 h	Room temperature	10.5	
	Enzyme assisted Extraction	1:20	0.5% 1:1:1 ratio of cellulase, papain, and pectinase	90 min	-	8.33	

2.13. Optimization of extraction parameters:

Statistical methods play a very essential role in science in technology. Experimental design must focus on various parameters influencing the process. To construct a proper experimental design the operation and process of a system should be studied well (Pashaei et al., 2020). Response Surface Methodology is one such experimental design used for optimizing an experiment. RSM is a statistical method which helps to optimize the process conditions with limited number of experimental trials. It enables the researchers to study the effects of the experimental factors and their interactions (Khuri & Mukhopadhyay, 2010). There are two different models in RSM. They are first degree model and second-degree models. Designs fitted for first degree models are called first order designs and designs fitted for second degree models are called as second order designs. The most used first order designs are 2k factorial (k- number of control variables) design, Plackett-Burman design (Mansouri et al., 2018), and simplex designs. The most commonly used second order designs are 3k factorial design, central composite design (Hang et al., 2011), and the Box-Behnken design (S. L. C. Ferreira et al., 2007). Plackett-Burman designs are used in screening to detect the influential factors of the experimental response (Mansouri et al., 2018). It is one factor at a time approach (OFAT) and this method is laborious, less capable of finding real optimum levels due to the existing interactions among the factors. Statistically designed experiments could be effective in this case. It also reduces the number of experiments and increases the process efficiency (El-Sheekh et al., 2016). Box-Behnken designs (BBD) comes under the class of rotatable second order designs based on three level incomplete factorial designs. Compared to other methods like central composite, Doehlert matrix (Cerqueira et al., 2021) and three-level full factorial design, BBD and Doehlert matrix methods are efficient. The efficiency of an experimental design can be defined as the number of coefficients in the estimated model divided by the number of experiments. The main advantage of BBD is that it

doesn't contain combinations of all factors simultaneously even at their highest or lowest levels. It is helpful in avoiding redundancy in the experiments (S. L. C. Ferreira et al., 2007).

The optimization of extraction using microwave assisted extraction, ultrasound assisted extraction, and enzyme assisted extraction generally focus on microwave power, ultrasonic power and enzyme dosage respectively to obtain optimum yield. Furthermore, the other parameters that chiefly govern the extent of extraction of polysaccharides include time, temperature, pH and solid to solvent ratio. The efficiency of extraction of polysaccharide mainly depends on solvent infiltration, dissolution, and diffusion of polysaccharides. Solid to solvent ratio decides the rate of solvent penetration into the plant cells which is the rate determining step in the extraction. Low solid to solvent ratio may result in improper solvent penetration and may result in low efficiency. And when the solid to solvent ratio is high it may cause dilution of polysaccharide. Temperature is another key parameter in the extraction of polysaccharides. Temperature plays an important role in breaking the cell wall and release of internal contents. When the temperature is low, the breakage of cells may not be regular and release of polysaccharides may be low. When the temperature is high, the extraction of polysaccharide maybe high but the structure of polysaccharide gets destroyed resulting in low bioactivity. Time decides the extraction efficiency of polysaccharides. When the time is less, the breakage of cells may not be regular and release of polysaccharides may be low. When the time is long, the extraction of polysaccharide maybe high but there are possibilities for reduction in the bioactivity of polysaccharides. Microwave power/ Ultrasonic power is the main parameter that directly affects the polysaccharide yield. Ultrasonic power helps in disrupting plant cell wall by cavitation (Hammi et al., 2020, Sorourian et al., 2020) and Microwave power by increasing the intracellular pressure and volatilization of internal contents of cell (Z. Hu et al., 2018,Rostami & Gharibzahedi, 2016). The exposure of plant samples to optimum conditions of microwave

power/ ultrasonic power is will ensure higher extraction yield without compromising bioactivity of extracted polysaccharides.

3. PURIFICATION PROCESS TO PURIFY POLYSACCHARIDES:

There are some non-polysaccharide components such as proteins, pigments and small molecule compounds in the process of extracting polysaccharides. Therefore, in the process of polysaccharide separation and purification, these non-polysaccharide impurities are removed first, and then the polysaccharide extraction is carried out. The crude polysaccharides precipitated may be either purified by using chromatographic techniques or by chemical treatment. Purity of a polysaccharide is very important as it influences the biological activity of them.

3.1. Degreasing of samples before extraction:

Degreasing is removal of fat and lipids from the samples used for extraction of polysaccharides and is usually performed by refluxing them with organic solvents like ethanol, acetone and petroleum ether (Surin et al., 2020). These organic solvents not only provide degreasing but also decolourization (J. Li & Huang, 2021).

3.2. Removal of pigments from polysaccharides:

Mostly polysaccharides may be associated with some pigments like tannins. These pigments are removed by activated carbon treatment, organic solvent treatment and hydrogen peroxide treatment (Zhan et al., 2020). Activated carbon treatment is employed to remove pigments like tannins but the major disadvantage of this method is that the loss of polysaccharide is high and the activated carbon might leach along with the polysaccharides affecting the quality (R. Chen et al., 2011). Hydrogen peroxide is suitable to remove pigments with unsaturated double bonds, and

aromatic groups. But it was reported that hydrogen peroxide damaged the structure of the polysaccharides and altered the bioactivity of the polysaccharides (Zhan et al., 2020).

3.3. Deproteinization process of polysaccharide:

Deproteinization is a very essential step in the purification of polysaccharides. Often proteins get coextracted with the polysaccharides. These proteins have to be removed to achieve the complete bioactivity of the polysaccharides (H. Cheng & Huang, 2018). The methods that are commonly used for deproteinization are treating the extracted polysaccharides with hydrochloric acid, trichloroacetic acid, calcium chloride, sodium hydroxide, proteolytic enzymes, Sevag method and Sevag-enzymatic combined method (X. Lin et al., 2019). Of the various methods of deproteination, loss of polysaccharides is very less in Sevag method but the rate of deproteinization is very less and hence repetitions are required (J. Li & Huang, 2021). In this method, organic solvents like n-butanol and chloroform are used to denature and remove proteins. As the process is repeated a lot of times, it takes long time and the toxicity of the organic solvents has to be considered (D. Y. Zhang et al., 2016). The use of Trichloroacetic acid to remove proteins though simple is very harsh and affects the structure of polysaccharides. Enzymatic deproteinization is highly specific and efficient as it causes only very less loss of polysaccharides (Zhan et al., 2020)

3.4. Membrane separation method:

Recently membrane separation techniques like dialysis and ultrafiltration are commonly used in polysaccharide separation and concentration (Zhan et al., 2020).

3.4.1. Dialysis:

In dialysis, semi permeable membrane of certain pore size is used to allow small molecules to permeate through it while larger molecules stay. This method is used to remove smaller carbohydrate units based on differences in pore size of membrane used (D. Y. Zhang et al., 2016). This method is very simple, green and imparts no damage to the polysaccharide structure (L. Chen et al., 2020).

3.4.2. Ultrafiltration:

Ultrafiltration uses molecular sieve principle and is a pressure driven separation process. Ultrafiltration can be operated continuously and doesn't impart any major changes on the polysaccharide structure (Zhan et al., 2020). Due to concentration polarization and membrane fouling the permeability may alter. Also some active polysaccharides may have high viscosity that may further block the membrane. This method is simple and rapid (Xie et al., 2014).

3.4.3. Fractional precipitation method:

Fractional precipitation involves step by step precipitation of polysaccharides in the increasing order of molecular weight upon increasing concentration of organic solvent like lower alcohol and ketones (Gong et al., 2018). This can also be done by adding salts resulting in salting out. The basic principle of salting out is that the solubility of polysaccharides differ according to salt concentration. Upon the addition of these salts the polysaccharides gradually precipitate. Like protein precipitation ammonium salts can also be used to precipitate acidic and neural polysaccharides extracted from plants (Zhan et al., 2020).

3.5. Separation of polysaccharides by column chromatography:

Column chromatography is used to purify the polysaccharides. Among the various kinds of chromatographic techniques, gel filtration chromatography and ion exchange chromatography are widely used.

3.5.1. Gel filtration chromatography:

In gel filtration chromatography, the column is filled with porous gel in three dimensional network structures (R. Chen et al., 2011; Pawlaczyk-Graja et al., 2019). The smaller molecules take a longer route by entering the pores in the matrix while the larger molecules passes through the void space and reaches the end of the column sooner than the smaller molecules thus creating a sieving effect. And the molecules get separated based on molecular weight (Y. Li, Qin, et al., 2020; X. Yang, Chen, et al., 2020).

3.5.2. Macroporous Resin Column Chromatography

In this technique the polysaccharides were separated and purified by selective adsorption of polysaccharides on the macroporous resin. The macroporous resin is of high stability and reusability (Y. Yang, Lei, et al., 2020). Here the structure of the polysaccharide remains undamaged. But the application of this method is limited due to its high cost and tedious regeneration of the column (Zhan et al., 2020).

3.5.3. Ion Exchange Column Chromatography

Separation of polysaccharides based on the ionic interaction with the column material is the principle of ion exchange chromatography (Gong et al., 2018). Ionic polysaccharides interact with the oppositely charged group in the column while the neutral polysaccharides flow out (J. Li & Huang, 2021). The separation is affected by flow rate, column height, elution solvent, inner diameter of column, and particle size. To achieve better separation several columns can be used simultaneously. The commonly used anion exchange columns were Diethyl Amino Ethyl (DEAE) cellulose column (R. Chen et al., 2011; Rostami & Gharibzahedi, 2017; Y. F. Zheng et al., 2016), Sephadex gel column (Ma et al., 2020; J. Wang et al., 2016; Y. F. Zheng et al., 2016) and agarose gel column (Popov et al., 2014).

3.6. Purification processes used in extraction of polysaccharides from plants:

Polysaccharides extracted from *Allium sativum* is deproteinated using different methods – HCl deproteinization, TCA deproteinization, NaCl deproteinization and $CaCl_2$ deproteinization and Cheng and Huang, 2018 compared the four deproteinization methods in means of efficiency of the process and the polysaccharide loss due to the treatment. And they concluded that $CaCl_2$ deproteinization was the best method among the four even though HCl method excelled in deproteinization, as it showed very low polysaccharide loss (H. Cheng & Huang, 2018). Crude polysaccharides from *Alpiniae oxyphyllae* (X. Yang, Yang, et al., 2020) and *Arctium lappa* (Y. yuan Jiang et al., 2019)were deproteinated using Sevag method (R. Zhu et al., 2019) and purified using DEAE Cellulose-52 column and Sephadex 100 column and obtained purified fractions of polysaccharides. Polysaccharides extracted from *Artemisia selengensis* were deproteinated using TCA method (Lau et al., 1985) and purified using DEAE Cellulose-52 column chromatography and two purified fractions were obtained. The molecular weights of the two fractions were found to be 125.4 and 184.1 kDa, respectively (J. Wang et al., 2016). Polysaccharide extracted from *Bellamya quadrata* was purified by Q sepharose fast flow and Sephacryl S-400 gel filtration column chromatography. Two different polysaccharide fractions of purified polysaccharides were obtained (Xiong et al., 2020). *Broussonetia papyrifera* crude polysaccharides were deproteinated using Sevag method. The deproteinated polysaccharides were purified using DEAE cellulose-52 and Sephadex 100 chromatography and three fractions were obtained (Han et al., 2016). *Camptotheca acuminata* crude polysaccharides were dissolved in deionized water and treated with α amylase to digest the starch present. The digested polysaccharides were subjected to Sevag treatment to remove the associated proteins. The deproteinated polysaccharides were further purified using DEAE cellulose-52 and Sephadex 100 chromatography (W. Hu et al., 2019). Polysaccharides extracted from *Chimonobambusa*

quadrangularis were redissolved in hot water and subjected to deproteinization by Sevag method for ten times. The deproteinated polysaccharides were dialysed (3000 Da) against water for 72 h (G. Chen, Chen, et al., 2019). *Lentinus edodes* crude polysaccharide was further purified using DEAE cellulose-52 and Sephadex 100 chromatography and two fractions were obtained which were used for further analysis(Y. ming Zhao et al., 2016). *Magnolia kwangsiensis* crude polysaccharides were purified using DEAE-52 cellulose column and Sepahdex G-100 column chromatography and two fractions were obtained – P2 and P3 (Y. F. Zheng et al., 2016).

The mulberry leaf polysaccharides were purified by three step procedures – deproteinization, dialysis and decolourization. Treatment with chloroform and isoamyl alcohol (Sevag method) to deproteinize and macroporous resin ADS-17 treatment to decolourize and remove the flavonoids and phenolic compounds present in the polysaccharide samples. Dialysis was performed against deionized water (D. Y. Zhang et al., 2016).

4. SUMMARY:

This review paper comprises different methods of extraction of polysaccharides from plants and its purification. Both advantages and disadvantages of the extraction methods were discussed. Technical aspects of those extraction methods and yield of polysaccharide extracted from different plants were compared. Out of all the methods used, Ultrasound assisted extraction is the most widely used method, as the yield of polysaccharide obtained is very high compared to other methods. This method is highly preferred as it is cost efficient and easy compared to other methods. Even though every method varies significantly from each other, the yield and purity mainly depend on the plant part used for extraction. And the purification process varies accordingly. The polysaccharide extracted from plants holds significant importance in terms of biological activity and application. As of now polysaccharides find their application in drug delivery systems, nanotechnology, prosthetic development, tissue regeneration, fabrication of

biomedical devices, food packaging industry, food preservation, and cosmetics. Any extraction and purification process should be capable of operating in large scale with optimum capital expense.

DECLARATION OF INTEREST:

None

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