MICROWAVE-ASSISTED EXTRACTION OF POLYSACCHARIDE FROM CINNAMOMUM CASSIA WITH ANTI-HYPERPIGMENTATION PROPERTIES

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by

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LIST OF SYMBOLS

A	Alpha
&	And
~	Approximately equal
B	Beta
°C	Degree Celsius
Δ	Delta
E	Epsilon
=	Equal
Λ	Lambda
<	Less than
>	More than
%	Percentage
±	Plus-minus
Σ	Sigma
×	Times

LIST OF ABBREVIATIONS

µg	Microgram
µL	Microliter
µm	Micrometer
3-PBA	3-Phenylbenzoic acid
ABS	Absorbance of sample
AC	Adenylate cyclase
ACN	Acetonitrile
ANOVA	Analysis of variance
ATP	Adenosine triphosphate
ATRA	All-trans retinoic acid
B:S	Buffer to sample
BBD	Box Behnken design
BSA	Bovine serum albumin
c-AMP	Cyclic adenosine monophosphate
CCD	Central composite design
cm^{-1}	Inverse centimeters
Cp	Number of the central points
CREB	cAMP-responsive element-binding protein
CuSO4	Copper (II) sulfate
Da	Dalton
DE	Degree esterification

DHI	5,6-Dihydroxyindole
DHICA	5,6-Dihydroxyindole-2-carboxylic acid
DNA	Deoxyribonucleic acid
DQ	DOPAquinone
EDTA	Ethylenediaminetetraacetic acid
EE	Erythemal effect spectrum
ES	Enzyme-substrate
F	Optimized for FRAP
FeCl3	Iron III chloride
FeSO4	Iron (II) sulfate
FRAP	Ferric reducing antioxidant power
FTIR	Fourier-transform infrared spectroscopy
g	Gram
GAC	Galacturonic acid content
GAE	Gallic acid equivalent
h	Hour
H2O	Water
HCL	Hydrochloric acid
HG	Homogalacturonan
HPLC	High-performance liquid chromatography
HQ	Hydroquinone
I	Holar intensity spectrum
i.e.	*Id est* (that is)

k	Number of factors
kDa	Kilo Dalton
KH_2PO_4	Potassium dihydrogen phosphate
L-DOPA	3,4-Dihydroxy-L-phenylalanine
M	Molar
MAE	Microwave-assisted extraction
MC1R	Melanocortin 1 receptor
ME	Maltose equivalent
mg	Milligram
min	Minute
MITF	Microphthalmia-associated transcription factor
MITF	Microphthalmia-associated transcription factor gene
ml	Milliliter
mM	Millimolar
MW	Molecular weight
NaOH	Sodium hydroxide
ND	Not detected
NKEA	National Key Economic Areas
nm	Nanometer
NS	Not selected
OH	Hydroxyl group
P	Mass of lyophilized polysaccharide
PAH	Phenylalanine hydroxylase

pH	Potential of hydrogen
PIH	Pregnancy-induced hypertension
PKA	Protein kinase A
PKC	Protein kinase C
PMP	1-Phenyl-3methyl-5pyrazolone
Qn	o-Dopaquinone
R1	Linearity
R2	Branching
R3	Branch size
RGI	Rhamnogalacturonan I
RGII	Rhamnogalacturonan II
ROS	Reactive oxidative species
rpm	Revolutions per minute
RRMW	Ratio of water to raw material
RSM	Response Surface Methodology
S	Mass of cinnamon powder
S	Optimized for SPF
s	Second
SPF	Sun protection factor
T	Optimized for anti-tyrosinase inhibitory activity
TAC	Total anthocyanin content
TAE	Tris-Acetate-EDTA
TCC	Total carbohydrate content

TFA	Trifluoroacetic acid
TPC	Total phenolic content
TPTZ	2,4,6-Tris(2-pyridyl)-s-triazine
TYR	Tyrosinase
TYRP-1	Tyrosinase-related protein 1
TYRP-2	Tyrosinase-related protein 2
U	International unit of enzyme activity
UAE	Ultrasound-assisted extraction
USA	United States of America
UVR	Ultraviolet radiations
v/v	Volume per volume
W	Watt
w/v	Weight per volume
w/w	Weight-to-weight
Y	Optimized for yield
α-MSH	Alpha-melanocyte-stimulating hormone

PENGEKSTRAKAN POLISAKARIDA YANG BERSIFAT ANTI-HIPERPIGMENTASI DARIPADA *CINNAMOMUM CASSIA* DENGAN BANTUAN PENDEKATAN GELOMBANG MIKRO

ABSTRAK

Polisakarida dengan sifat anti-hiperpigmentasi tidak pernah dilaporkan. Kajian semasa telah mengekstrak polisakarida daripada kulit kayu *Cinnamomum cassia* dengan menggunakan pendekatan bantuan gelombang mikro, dan mengoptimumkan parameter pengekstrakan (iaitu kuasa gelombang mikro, masa penyinaran dan nisbah penimbal kepada sampel (B:S)) berdasarkan rekabentuk Box-Behnken bagi mendapatkan polisakarida dengan aktiviti anti-hiperpigmentasi, aktiviti antioksidan, faktor perlindungan matahari (SPF) serta hasil pengekstrakan yang tinggi. Model-model telah berjaya dibangunkan, dan maklum balas yang dioptimumkan ialah: (a) aktiviti perencatan monophenolase=97.5% dan (b) aktiviti perencatan diphenolase=99.4% dalam kondisi: kuasa=224.7 W, masa=1.1 min, and nisbah B:S =31.7 mg/ml; (c) kuasa antioksidan penurun ferik=4.4 mM dalam kondisi: kuasa=224.7 W, masa=1.1 min, and nisbah B:S =31.7 mg/ml; (d) faktor perlindungan matahari (SPF)=6.1 dalam kondisi: kuasa=400 W, masa=3 min, and nisbah B:S ratio=30 mg/ml; (e) hasil pengekstrakan=13.7% dalam kondisi: kuasa=592.1 W, masa=2.5 min, and nisbah B:S ratio=31.9 mg/ml. Pektik-polisakarida yang diekstrak adalah rendah dalam berat molekul dan tahap pengesteran. Penyumbang utama untuk aktiviti anti-hiperpigmentasi, antioksidan dan SPF adalah pektik-polisakarida dan fenolik yang berada pada cawangan polisakarida. Melalui kajian kinetik, corak perencatan campuran yang ditunjukkan oleh polisakarida kayu manis

melalui plot Lineweaver–Burk. Juga, polisakarida dapat mengelat kuprum, dimana ia boleh merencat aktiviti tyrosinase secara tidak langsung. Oleh demikian, dicadangkan bahawa polisakarida daripada *C. cassia* boleh menjadi penyelesaian alternatif bagi rawatan hiperpigmentasi kulit.

MICROWAVE-ASSISTED EXTRACTION OF POLYSACCHARIDE FROM CINNAMOMUM CASSIA WITH ANTI-HYPERPIGMENTATION PROPERTIES

ABSTRACT

Polysaccharides with anti-hyperpigmentation properties have not been reported elsewhere. The current study extracted the polysaccharides from *Cinnamomum cassia* bark using microwave-assisted approach, and optimized the extraction parameters (i.e. microwave power, irradiation time and buffer-to-sample (B:S) ratio) based on Box-Behnken design to obtain polysaccharides with high anti-hyperpigmentation activities, antioxidant activity, sun protection factor (SPF) as well as the extraction yield. The models were successfully developed, and the optimized responses were: (a) monophenolase inhibitory activity=97.5% and (b) diphenolase inhibitory activity=99.4% under condition: power=224.7 W, time=1.1 min, and B:S ratio=31.7 mg/ml; (c) ferric reducing antioxidant power=4.4 mM under condition: power=224.7 W, time=1.1 min, and B:S ratio=31.7 mg/ml; (d) sun protection factor (SPF)=6.1 under condition: power=400 W, time=3 min, and B:S ratio=30 mg/ml; (e) extraction yield=13.7% under condition: power=592.1 W, time=2.5 min, and B:S ratio=31.9 mg/ml. The pectic-polysaccharides were in low molecular weight and degree of esterification. The main contributors for the anti-hyperpigmentation activity, antioxidant and SPF were the pectic-polysaccharide itself and some of the phenolics present in the branch side of the polysaccharide. Through kinetic study, mixed inhibition pattern was shown by the cinnamon polysaccharide based on the Lineweaver–Burk plot. Also, the polysaccharides were able to chelate copper, which could

indirectly inhibit the activity of tyrosinase. It was therefore proposed that the polysaccharides from *C. cassia* could be an alternative solution for skin hyperpigmentation treatment.

CHAPTER 1

INTRODUCTION

1.1 Background of the study

The most common type of cinnamon in local markets is cinnamon cassia (*Cinnamomum cassia*), which is widely accessible and simple to acquire because it is less expensive than other cinnamon. It is an evergreen aromatic plant species endemic to tropical areas, particularly in east and southeast Asia; China is the main producer (Chen *et al*., 2021). It is a medicinal herb that has different pharmacological activities such as an antioxidant, antidiabetic, neuroprotective agent, and anticholesterol and is regarded as an alternative natural medicine (AlMohaimeed *et al*., 2021; Nwanade *et al*., 2021) and that due to the presence of different active components such as coumarin, cinnamic acid, and cinnamaldehyde (Cha *et al*., 2019). A small community from northern region of Malaysia has been using this plant to treat skin issues. Therefore, the current was to explore the potential of polysaccharide from cinnamon cassia in skin healthcare.

The extraction of the active components posed a great challenge for the researchers, as their goal was to obtain the highest yield and the highest activity. Researchers have explored for strategies to limit the consequences of deterioration and reduce the use of solvents using the modern techniques such as microwave assisted extraction, ultrasonic assisted extraction, Soxhwave, etc. (Gupta, Naraniwal, & Kothari, 2012). Therefore, the current study was about to use microwave-assisted extraction technique to extract polysaccharide from cinnamon cassia.

Hyperpigmentation consider as one of the important disorders in the skin related to lack control of tyrosinase enzyme due to either extrinsic (UV light) or intrinsic (Melanocytes' reaction to ultraviolet radiation is influenced by hormonal regulation) factors. Cinnamon cassia has been shown, through its component cinnamaldehyde, to inhibit hyperpigmentation by inhibiting the enzyme tyrosinase (Ismail, Shahidan, & Ponnuraj, 2021). Hence, extracted polysaccharide from cinnamon cassia might have the same potential.

1.2 Problem statement

Skin conditions are among the most frequent disorders that have an adverse psychological impact on humans, such as increasing anxiety and depression, which can have a negative impact on quality of life (Wheeler *et al.*, 2022). Hyperpigmentation is one of the most important skin disorders, which increases the production of melanin associated with a lack of control of the tyrosinase due to oxidative agents or UV light; they usually appear as spots or dark patches on the skin. Some therapeutic medications are there, which helped to reduce these cases, but most of them are either radiation- or chemical-dependent drugs, which are unsafe for humans. For example, kojic acid, arbutin and hydroquinone are associated with harmful side effects including contact dermatitis, irritation and even genotoxicity and carcinogenicity (Nohynek *et al.*, 2004; Westerhof, & Kooyers, 2005). These drawbacks, therefore, urge the search for a cheaper, safer, and more natural alternative anti-hyperpigmentation agent with high efficacy (Zolghadri *et al.*, 2019). The fact that cinnamon is one of the plants with a high resistance to free radicals since it consists of useful components (refer to Section 2.1), leads us to consider using natural sources as an efficient type of treatment for skin disorders. Yet, none of the recent studies

mentioned polysaccharides as the primary component of cinnamon, which made this research's focus on polysaccharides important considering that the polysaccharides have a variety of functions like antioxidants and antimicrobials (refer to Section 2.2.3). Below are the research questions raised:

a) How do the extraction parameters, such as microwave power, extraction time, type of extraction buffer and cinnamons-to-buffer ratio affect the anti-hyperpigmentation properties (i.e., tyrosinase inhibitory, antioxidant, and UV protection activities) of the extracted polysaccharide?
b) How does the extracted polysaccharide contribute to the anti-hyperpigmentation, antioxidant, and UV protection properties?

1.3 Hypothesis

In this study, tyrosinase is the targeted enzyme that the polysaccharides should inhibit so that the melanogenesis process or the hyperpigmentation could be suppressed. The novelty of this research is focusing on the mechanistic of cinnamon polysaccharides with anti-tyrosinase, antioxidant, and UV protection activities to attenuate skin hyperpigmentation. In this case of study, cinnamons, which are aromatic condiments/spices that could be harvested from the inner bark of the *Cinnamomum* tree, are suggested as alternative sources for anti-hyperpigmentation agent because of its medicinal properties. To our knowledge, researchers were only focusing on the research of its essential oil and its principal component, cinnamaldehyde, as well as other constituents, such as cinnamate, cinnamic acid, p-cymene, and eugenol (Almatroodi *et al.*, 2020; Yang, Zhao, & Jiang, 2008). In addition, we also found that cinnamons contain large

amounts of bioactive polysaccharides during the preliminary experiment. It was hypothesized that the polysaccharide haves the potential in binding to tyrosinase that caused the physical obstruction that prevented the substrate from entering to the active sites of tyrosinase. Also, polysaccharide could contribute to other activities such as antioxidant and UV protection activities.

1.4 Significance of the study

Under National Key Economic Areas (NKEA)-Agenda 9- Health care, the present proposed study contributes to the development of drug delivery experts and room for beneficial polysaccharide therapeutics and approaches that are developed based on a fundamental understanding of the anti-hyperpigmentation mechanism based on the tyrosinase inhibitory activity, antioxidant activity, and UV protection effect, which highlights the use of natural products as secure alternatives to radioactive and chemical products. It should be noted that the interaction between polysaccharide and tyrosinase has not been reported elsewhere. No other research has been reported about the polysaccharides in cinnamons or their aforementioned activities or their mechanism. From the patent search, we did not find any similar products neither from cinnamon nor polysaccharide as well. Therefore, it is believed that this study is essentially novel.

1.5 Objectives

The aim of this study was to investigate the anti-tyrosinase, antioxidant, and UV protection effects of the bioactive polysaccharides derived from cinnamons that can be potentially used as a skincare product. This study has 2 specific objectives:

a) To optimize the effects of extraction buffer concentration, cinnamon-to-buffer ratio, microwave power, and extraction time that influence the anti-hyperpigmentation properties of cinnamon polysaccharide and to optimize the aforementioned extraction parameters using Box Behnken Design.
b) To investigate the chemical and structural properties of the extracted polysaccharide that contribute to the anti-hyperpigmentation properties which include the mechanism and mode of tyrosinase inhibition.

CHAPTER 2

LITERATURE REVIEW

2.1 Cinnamon

Cinnamon is one of the important spices and is obtained from the dried inner bark of green trees belonging to the genus *Cinnamomum* (Rana *et al*., 2021). There are many types of cinnamon, and they are distributed in different regions, such as cinnamon Ceylon (true cinnamon), cinnamon *cassia* (Chinese cinnamon), cinnamon *burmannii* (Indonesian cinnamon), *C. loureiroi* (Vietnamese cassia), and cinnamon *citriodorum* (Malabar cinnamon). China and Indonesia account for 70% of the production volume of cinnamon in East and Southeast Asia, with Indonesia accounting for 40% and China for 30%. Other production countries are Sri Lanka, India, Vietnam, Seychelles, and Madagascar (Nwanade *et al*., 2021; Chen *et al*., 2021).

2.1.1 Cinnamon cassia

Cinnamon cassia (*Cinnamomum cassia*) is the most found cinnamon in the local markets, and it is available to all and easy to obtain because it is lower in price compared to other cinnamon. It belongs to the *Lauraceae* family, and it is an evergreen aromatic plant species, which is native to tropical regions, especially in east and southeast Asia; China is the major country producers (Nwanade *et al*., 2021 & Chen *et al*., 2021). It is a medicinal plant and is considered as alternative natural medicine that has traditionally been used as an antioxidant, antidiabetic, and neuroprotective agent (AlMohaimeed *et al*., 2021). Therefore, it was selected as the study material in this study.

2.1.2 Recent studies on cinnamon cassia

Cinnamon cassia is very rich in various vital components, which translates into many benefits and important activities. The main component of the oil from cassia bark and leaf is cinnamaldehyde. This component differs in different parts of the plant and the plants that originated from different regions (Zachariah & Leela, 2006). Table 2.1 shows the recent studies on cinnamon cassia. Overall, these studies were focusing on the different extraction techniques, such as conventional maceration, hydrodistillation, microwave-assisted, and ultrasound-assisted extraction approaches to obtain essential oil and targeted compounds such as coumarin, cinnamic acid, and cinnamaldehyde. It could be observed that the cinnamaldehyde is still the most commonly studied compound, followed by coumarin. It should also be noted that polysaccharide/pectin from the bark is attracting the attention of the researchers for their biological activities. For examples, anti-tumor from the root bark pectin of *Aralia elata* (Yong-Gang *et al*., 2020), immunomodulating effects from the bark of *Cola cordifolia* (Austarheim *et al*., 2012), as well as anticoagulant, antiplatelet, and antithrombotic activities from *Caesalpinia ferrea* stem barks (de Araujo *et al*., 2021). Yet, cinnamon bark polysaccharide/pectin has not been explored. Therefore, it is worth investigating the potential of cinnamon bark polysaccharide/pectin.

Table 2.1 Different extraction methods to extract different active component from cinnamon cassia.

Extraction method	Active component	Activity	Reference
Ultrasound-assisted hydrodistillation extraction and conventional hydrodistillation	Monoterpenes (Butylbenzene), oxygenated monoterpenes (2-methoxycinnamaldehyde) Sesquiterpene hydrocarbons (Copaene), oxygenated sesquiterpenes (caryophyllenyl alcohol), other oxygenated compounds (trans-cinnamaldehyde)	NS	Chen *et al.* (2021)
Subcritical extraction	Coumarin, cinnamic acid, cinnamaldehyde, cinnamyl alcohol	NS	Cha *et al.* (2019)
Subcritical n-butane and ethanol extraction	(E)-cinnamaldehyde and coumarin	Antibacterial	Liang *et al.* (2019)
Microwave-assisted extraction, ultrasound-assisted extraction and reflux extraction	Cinnamic acid and cinnamaldehyde	NS	Lee *et al.* (2018)
Microwave-assisted hydrodistillation and conventional hydrodistillation	Oxygenated monoterpenes (eugenol), sesquiterpene hydrocarbons (alpha-Cubebene), oxygenated sesquiterpenes (caryophyllene oxide), other oxygenated compounds (trans-cinnamaldehyde)	Cytotoxicity	Jeyaratnam *et al.* (2016)
Classical solvent extraction, ultrasonication, maceration and shaking.	Phenolic compounds (sinapic and cinnamic acids. Caffeic, vanillic acid, and 3,4-dihydroxybenzaldehyd)	Antioxidant	Dvorackova *et al.* (2015)
Supercritical carbon oxide and water.	Cinnamaldehyde	Antioxidant and prooxidant	Sia *et al.* (2013)

Extraction method	Active component	Activity	Reference
Ethanol and supercritical fluid extraction	Phenolics and flavonoids	Antioxidant	Yang *et al.* (2012)
Hydrodistillation	Trans-cinnamaldehyde, glycerol 1-methyl ether, o-methoxy cinnamaldehyde, alkenes, alkanes, alcohols, aldehydes, amines, carboxylic acids, ethers, esters, and ketones,	NS	Wang *et al.* (2011)
Solid phase microextraction	1,2,3,4,4a,5,6,8aoctahydro-7-methyl-4-methylene-1-(1-methylethyl)-naphthalene, 1,2,3,4a,5,8,8a-hexahydro-4,7-methyl-1-methylene-1-(1-methylethyl)-naphthalene and copaene		
Maceration (methanol) and distillation	(E)-cinnamaldehyde	Acaricidal	Nwanade *et al.* (2021)

2.2 Polysaccharide

Polysaccharides are large molecules made of common saccharide monomers and galacturonic acid as the building blocks (Han *et al.*, 2022). Based on the number of repeating monomeric units, these monomeric units bind with each other by a glycosidic linkage to create a large non-biodegradable natural polymer or glycan (polysaccharide). Polysaccharides can be classified based on their structure, chemical property, or monomeric unit. For example, linear polysaccharide or branched polysaccharide, neutral or acidic polysaccharide, and heteroglycan or homoglycan. In this study, pectic-polysaccharide or pectin is the targeted component that was extracted. Pectin is one of the heterogenous anionic polysaccharides (Zhang *et al.*, 2022) and it is widely present in cell

wall of higher plants, and it is the highest natural water-soluble dietary fiber (Hou *et al.*, 2022). Pectin is a complex polysaccharide with residues of esterified 1-4-linked α-D-galacturonic acid (Ibraheem *et al.*, 2022) and which contains homogalacturonan (HG), rhamnogalacturonan I (RGI), and rhamnogalacturonan II (RGII) (Hou *et al.*, 2022).

2.2.1 Pectic-polysaccharide sources

The polysaccharide can be found in different plant sources as it is made up of more than 80% (dry weight basis) of all plant material (BeMiller, 2018). Table 2.2 shows the recent studies on different sources of pectic-polysaccharides. It could be observed that fruits and their waste were the focus of the studies and contributed 61.4% in the recent pectic-polysaccharide studies, followed by vegetables (13.6%) and their wastes (4.5%) (Fig. 2.1). Among the fruits and their waste are pineapple peel waste, persimmon fruit, cocoa pods husk, olive, strawberries, apple pomace, and pomelos (Table 2.2). Whereas, the studied vegetables or their wastes were okra, beet pulp, sweet potato, carrots, and tomatoes. It should be noted that so far only two herbs (i.e., bay tree and *Flos Magnoliae*) were recorded in the polysaccharide research. In addition, plant bark is rarely studied; therefore, cinnamon bark (refer to Section 2.1) is considered a novel source of polysaccharides and it is worth for investigation in this project.

Table 2.2 Different reported sources of pectic-polysaccharides

Source type	Source	Reference
Fruit waste	Pineapple peel waste	Shivamathi *et al*. (2022)
Fruit	Persimmon fruit (*Diospyros kaki* Thunb)	Méndez *et al*. (2022)
Fruit and fruit waste	Citrus pectin and stalk pectin	Çavdaroğlu & Yemenicioğlu. (2022)
Herb	*Flos Magnoliae*	Wang *et al*. (2022)
Fruit	Apple pomace	Zhang *et al*. (2022)
Fruit waste	Cocoa pods husk	Valladares-Diestra *et al*. (2022)
Fruit	Fresh olive fruits	Bermúdez-Oria *et al*. (2021)
Fruit	Four citrus cultivars	Hu *et al*. (2021)
Seed waste	The flaxseed by-product (cake)	Ahmad *et al*. (2021)
Fruit	Fresh cantaloupe fruits	Kazemi *et al*. (2021)
Flower	Fresh sunflower heads	Ma *et al*. (2021)
Herb waste	Bay tree pruning waste	Rincón *et al*. (2021)
Fruit	Fresh strawberries (*Fragaria* × *ananassa*), blackberries (*Rubus fruticosus* L.), raspberries (*Rubus idaeus* L.), redcurrants (*Ribes rubrum*)	Muñoz-Almagro *et al*. (2021)
Seed	*Vitis vinifera* grape seed	Priyadarshi *et al*. (2022)
Vegetable	Fresh okra pods	Ma *et al*. (2021)
Fruit waste	*Annona squamosa* fruit waste	Shivamathi *et al*. (2019)

Source type	Source	Reference
Nut	Fresh green walnuts	Asgari *et al*. (2020)
Fruit waste	Banana (*Musa sapientum* L) peel	Phaiphan *et al*. (2020)
Fruit waste	Black mulberry pulp waste	Khodaiyan & Parastouei (2020)
Vegetable waste	Beet pulp	Elizaryev *et al*. (2020)
Fruit Waste	*Opuntia robusta* peel	Mota *et al*. (2020)
Fruit	Hawthorn wine pomace	Sun *et al*. (2020)
Vegetable	Industrial potato pulp	Arrutia *et al*. (2020)
Fruit	Pomelos	Li *et al*. (2020)
Fruits	Chardonnay grape pomace	Colodel *et al*. (2020)
Vegetable	Sweet potato (Xushu 18)	Arachchige *et al*. (2020)
Bean	Coffee pulp	Reichembach *et al*. (2020)
Fruit Waste	Lime peel	Rodsamran *et al*. (2019a)
Fruit Waste	Fresh pineapple peel	Rodsamran *et al*. (2019b)
Fruit	Common fig (*Ficus carica* L.)	Gharibzahedi *et al*. (2019)
Vegetable	Carrots (*Daucus carota* L. var. Nantes)	Idrovo Encalada *et al*. (2019)
Fruit	Mangosteen fruits	Wathoni *et al*. (2019)
Fruit Waste	Ponkan (*Citrus reticulata* Blanco cv. Ponkan) peel	Colodel *et al*. (2018)
Vegetable	Ripe pumpkin C. maxima D. var. Cabello de Ángel	Torkova *et al*. (2018)

Source type	Source	Reference
Fruit	Pomegranate	Ahmadi Gavlighi *et al*. (2018)
Fruit	Pomelo (*Citrus grandis* (L.) Osbeck) peels	Liew *et al*. (2018)
Fruit	Different varieties of pink/red and citric combinations of white grapefruits (*Citrus paradisi* (Mac.)).	La Cava *et al*. (2018)
Leaves	*Suaeda fruticosa* (L.) Forssk leaves	Mzoughi *et al*. (2018)
Fruit Waste	Jackfruit waste	Xu *et al*. (2018)
Fruit	Ripe fruits of acerola (*Malpighia emarginata*)	Klosterhoff *et al*. (2018)
Root	Ginseng roots	Jiao *et al*. (2014)
Vegetable	Tomatoes	Kapoor & Dharmesh (2016)
Vegetable waste	Sugar beet pulp	Lv *et al*. (2013)

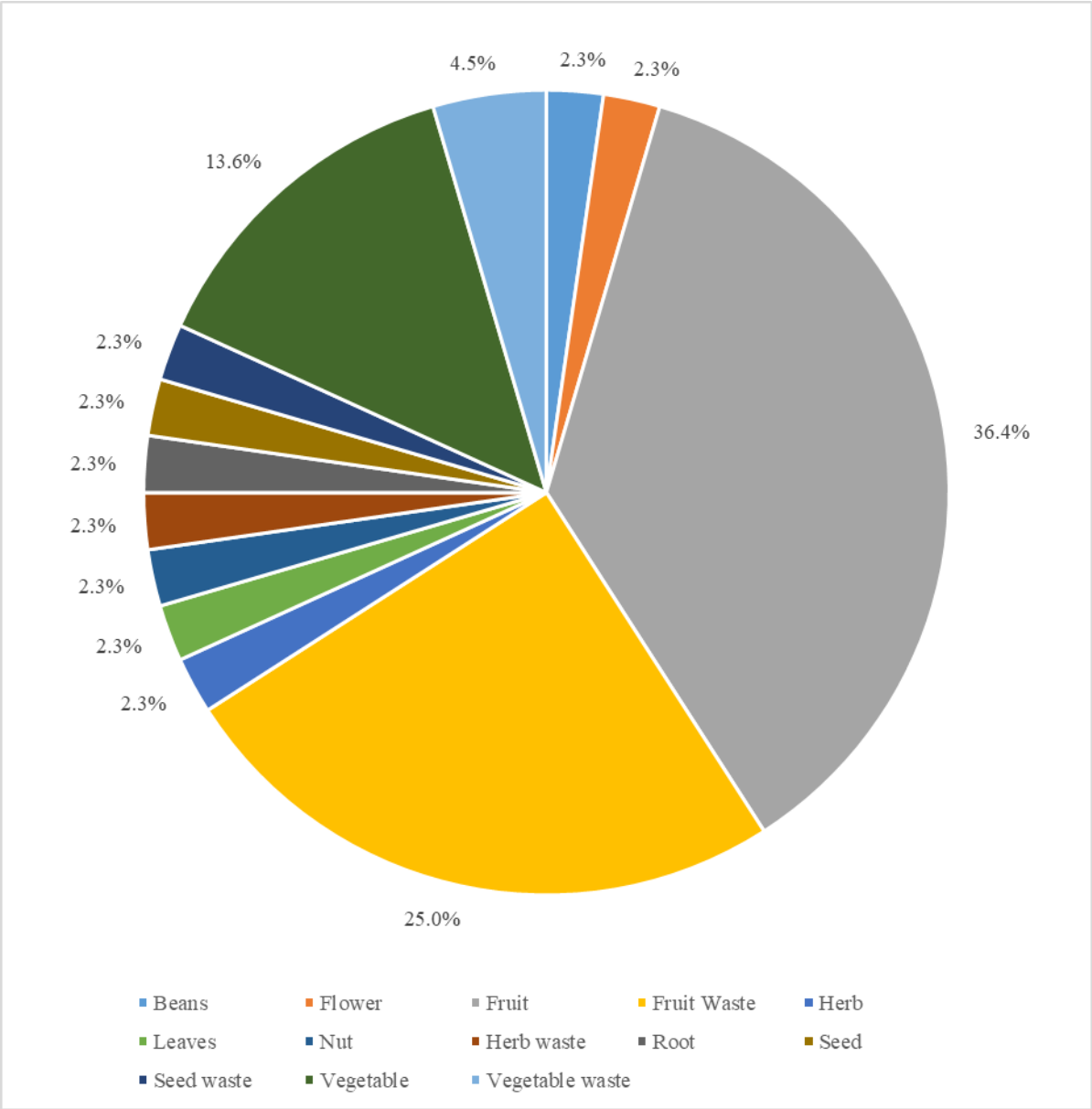

Figure 2.1 Percentages of pectic-polysaccharide source type reported in the literature.

Note: An estimated percentage was obtained for this study after a thorough review of more than 50 research papers from different databases.

2.2.2 Polysaccharide extraction techniques

Extraction methods differ according to their mechanisms. In general, two classifications, i.e., conventional extraction methods and non-conventional extraction methods, have been introduced. Conventional extraction methods are based on the type of

solvents, as each solvent is directed to a specific type of active component. Some examples of conventional extraction methods are solid-liquid extraction or maceration using water or acids (Colodel *et al*., 2020; de Andrade Vieira *et al*., 2022; Shang *et al*., 2019). Table 2.3 shows the recent reported extraction techniques used to extract polysaccharides from different sources. It could be observed that maceration is still the most common (~41.8%, Fig. 2.2) technique used by the researchers. Having said that, previous studies have shown that these approaches require long extraction time and high amount of solvents with low extraction efficiency (de Andrade Vieira *et al.*, 2022). Often these requirements are considered as a disadvantage.

Table 2.3 The different extraction methods used to extract polysaccharides.

Extraction method	Pectin	Reference
Ultrasound-assisted extraction (UAE)	Pineapple peel pectin	Shivamathi et al. (2022)
Maceration (Aqueous extraction)	Persimmon fruit pectin	Méndez et al. (2022)
Maceration (acid extraction)	Citrus pectin and stalk pectin	Çavdaroğlu & Yemenicioğlu. (2022)
Hot-compressed water extraction	*Flos Magnoliae* pectin	Wang *et al*. (2022)
Subcritical water extraction	Apple pomace pectin	Zhang et al. (2022)
Citric-acid-assisted hydrothermal pretreatment	Cocoa pod pectin	de Souza Vandenberghe et al. (2022)
Maceration (acid extraction)	Olive fruit pectin	Bermúdez-Oria et al. (2021)

Manosonication assisted extraction	Citrus pectin	Hu et al. (2021)
Microwave-assisted extraction (MAE)	Cantaloupe fruit pectin	Kazemi et al. (2021)
Maceration	Sunflower head pectin	Ma et al. (2021)
Sequential subcritical water extraction	Bay tree pectin	Rincón et al. (2021)
Maceration with/without UAE or enzymatic extraction	Strawberries pectin, blackberries pectin, raspberries pectin, redcurrant pectin	Muñoz-Almagro et al. (2021)
Enzyme-assisted acidic extraction	Butternut squash pectin	Milošević et al. (2022)
Maceration (water and acid extractions)	Okra pod pectin	Ma et al. (hun2021)
UAE	*Annona squamosa* fruit peel pectin	Shivamathi et al. (2019)
MAE	Green walnut pectin	Asgari et al. (2020)
MAE and UAE	Banana peel pectin	Phaiphan et al. (2020)
MAE	Black mulberry pulp pectin	Khodaiyan & Parastouei (2020)
Maceration (acid extraction)	Beet pulp pectin	Elizaryev et al. (2020)
MAE and Maceration	*Opuntia robusta* peel pectin	Mota et al. (2020)
Maceration (acid extractions) Enzymatic method and MAE	Hawthorn wine pomace pectin	Sun et al. (2020)
MAE	Potato pulp pectin	Urrutia et al. (2020)
Enzymatic pretreatment and maceration (acid extraction)	Pomelo pectin	Li et al. (2020)

Maceration (acid extraction)	Chardonnay grape pomace pectin	Colodel et al. (2020)
UAE and MAE	Sweet potato(Xushu18) pectin	Arachchige et al. (2020)
Maceration (acid extraction)	Coffee (*C. arabica* L) pulp pectin	Reichembach et al. (2020)
MAE	Lime peel pectin	Rodsamran et al. (2019a)
Maceration (acid extraction) MAE	Pineapple peel pectin	Rodsamran.et al. (2019b)
Maceration (acid extraction) and UAE-MAE	Common fig (*F. Carica* L.) fruit pectin	Gharibzahedi et al. (2019)
UAE	Carrots (*Daucus carota* L. var. Nantes) pectin	Idrovo Encalada et al. (2019)
Maceration (acid extraction)	Mangosteen fruit pectin	Wathoni et al. (2019)
Maceration (acid extraction)	Ponkan peel pectin	Colodel et al. (2018)
Cavitation facilitated method	Pumpkin pectin	Torkova et al. (2018)
Enzymatic extraction and Maceration (acid extraction)	Pomegranate pectin	Ahmadi Gavlighi et al. (2018)
Subcritical water extraction	Pomelo peel pectin	Liew et al. (2018)
Maceration (acid extraction) and thermosensation extraction	Citric × white grapefruits pectin	La Cava et al. (2018)
UAE	*Suaeda fruticosa* (L.) Forssk leaf pectin	Mzoughi et al. (2018)
UAE-MAE and maceration (acid extraction)	Jackfruit peel pectin	Xu et al. (2018)
Maceration (water extraction)	Acerola (*Malpighia emarginata*) fruit pectin	Klosterhoff et al. (2018)
Maceration (water extraction)	Ginseng pectin	Jiao et al. (2014)

Maceration (acid extraction)	Tomato pectins	Kapoor & Dharmesh. (2016)
Maceration (water extraction) then enzymatic treatment	Sugar beet pulp pectin	Concha et al. (2013)
Maceration (water extraction with pH adjustment)	Sugar beet pulp pectin	Lv et al. (2013)

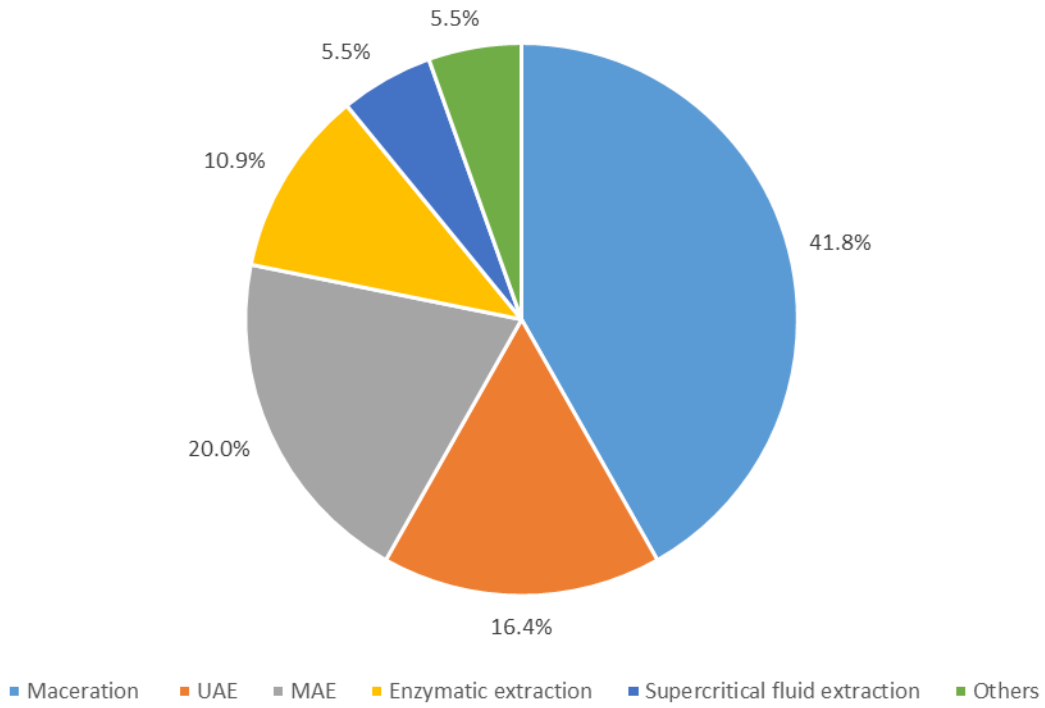

Figure 2.2 Percentage of extraction methods used to extract polysaccharides.

Hence, unconventional methods are getting their popularity. Unconventional extraction methods are modern methods, which are designed to be able more stable and

efficient for extracting bioactive components from plants (de Andrade Vieira *et al*., 2023). The utilization of unconventional extraction methods for the extraction of polysaccharides has several benefits, including quick extraction time, low energy and solvent use, and high extraction efficiency and yield (de Andrade Vieira *et al*., 2023). Among the techniques are supercritical fluid (Jafari, Zandi & Ganjloo, 2022), ultrasonic assisted extraction (UAE) and microwave assisted extraction (MAE) (de Andrade Vieira *et al*., 2023). In fact, Table 2.3 shows that MAE is the most favorable modern technique. MAE, which was therefore used in this project, is depending on the thermal radiation. The direct effect of microwaves on molecules by ionic conduction and dipolar enhances the heating of the irradiation medium, which breaks the matrix cell wall, and encourages the spread and solubility of polysaccharides in the solvent (de Andrade Vieira *et al*., 2023). In general, three main MAE extraction parameters should be taken into consideration: power (W), time (min), and ratio between sample and solvent (mg/ml), Table 2.4 shows the effects of these MAE parameters on the yield, antioxidant and anti-tyrosinase activities of polysaccharides and other extracts. It can be observed that the yield increased in different samples at a certain level of the extraction parameter and then decreased when above certain level. It could be explained that the increase in time works on the accumulation of heat (under the microwave radiation) in the extraction solution, which enhances the production of polysaccharides. On the other hand, polysaccharides may break down as a result of prolonged exposure to the microwave field, which causes a drop in production. The ratio between the solvent and the raw material depends on the type of solvent usually used and this is due to the solvent's ability to dissolve polysaccharide and its dielectric properties, where the yield increases with the increase in the ratio when it reaches a certain limit (Zheng *et al*., 2011). The power helps to lyse the cell wall which increases the extraction

efficiency and yield (Senthilkumar *et al*., 2022). However, antioxidants increase when the amount of energy used decreases. When more energy is consumed, the system heats up more and affects the antioxidants, like polyphenols. Also, the temperature should be at a lower level to obtain the highest antioxidant activity. On the other hand, antioxidant activity increases when the ratio increases from 13 to 47 mg/ml; but if the ratio increases too much, the antioxidant begin to decrease because the further increased concentration of the solvent causes more energy to be absorbed which means less energy around the solution and reduces the ability of the plant material to absorb microwaves, resulting in less effective extraction and lower polyphenol content in the extract. (Milutinović *et al.*, 2015). Studies have shown that the activity of anti-tyrosinase increases with the use of low energy and time, also it was shown that the activity increases with the use of a higher ratio (i.e., 30–40:1 ml/mg) (Kumar *et al.*, 2019). The most important advantage of this method is the rapid process, and there is no requirement to pre-treat the sample. However, like UAE, the high temperature could cause thermolabile substances to degrade and may have low extraction selectivity (Rodríguez-Mena *et al*., 2022). Therefore, optimization of the parameters is required to achieve the desired properties or yield.

Table 2.4 Effect of different parameters on the yield, antioxidant activity, and anti-tyrosinase activity from polysaccharide and other active components.

Source of polysaccharide and other extracts	Parameters	Effects of parameters on the responses	References
Annona reticulata fruit	• Ratio of water to raw material (RRMW): 1:40, 1:50, 1:60 • Extraction time: 30 to 60 min	• Improve the extraction yield of polysaccharides when increasing RRMW and time.	Senthilkumar *et al*. (2022)
	• Microwave power: 70 to 150 W	• Increasing microwave power initially results in increased polysaccharide extraction rates. As the microwave power was increased, the extraction rate slowed.	

Source of polysaccharide and other extracts	Parameters	Effects of parameters on the responses	References
Pumpkin (*Cucurbita pepo L.*)	• Extraction temperature: 50 to 90 ℃	• When the extraction temperature was raised, the production of polysaccharides increased and reached its peak at 70 ℃. It then starts to progressively reduce after that.	Zheng *et al.* (2011)
	• Time: 10 to 50 min	• The polysaccharides yield increased with extraction time, peaked at 30 min, and immediately decreased.	
	• Liquid/solid ratio: 10 to 50 ml	• The yield of polysaccharides increased as the ratio increased and reached its maximum of 20 ml; however, it then started to decrease.	
Opuntia Ficus-indica cladodes	• Extraction time: 1 to 7 min	• With longer extraction times the yield of polysaccharides increased quickly.	Felkai-Haddache *et al.* (2016)
	• Microwave power: 500 to 900 W	• The polysaccharide yield is increased by increasing the microwave power level.	
	• Ratio Mixed cladode-water/ material: 1 to 5 mL/g	• The yield started to increase from 1 to 4 mL/g after that the line begins to settle	

Source of polysaccharide and other extracts	Parameters	Effects of parameters on the responses	References
Ulva meridionalis and *Ulva ohnoi*	• Reaction temperature: 100 to 180 ℃	• The yield has begun to increase from 100 to160 °C. • The chemical compositions of ulvan were almost stable below 160 °C, but they started to decompose at 180 °C. • The solubilization rate increased with an increase in the temperature.	Tsubaki *et al.* (2016)
Monostroma latissimum	• Reaction temperature: 100 to 180 ℃	• The solubilization rate increased with an increase in the temperature. • The yield increased from 100 to 140 °C, and then they drastically decreased above 160 °C.	Tsubaki *et al.* (2016)
Mulberry leaves	• Weight of the sample: 6 to 24 g • Microwave power: 50 to 250 W • Extraction time: 5 to15 min	• The yield of polysaccharides increased from 6 to 20 g, after that it decreased. • The yield of polysaccharides increased from 50 to 250 W. • The yield of polysaccharides increased up to 13 min, beyond 13 min of extraction time shows a negligible effect on the yield.	Thirugnanasambandham *et al.* (2015)

Source of polysaccharide and other extracts	Parameters	Effects of parameters on the responses	References
Cyphomandra betacea	• Ratio of water to raw material: 1:40 to 1:60	• When the ratio increased, the yield increased especially at high extraction time.	Sivakumar & Ruckmani (2016)
	• Microwave power: 300 to 400 W	• It showed here that when the energy is increased, the rate of extraction will increase, and thus the yield increases, but after this and when the energy is excessively increased, the rate of extraction begins to decrease.	
	• Extraction temperature: 50 to 70 ℃	• The yield of polysaccharides was increased with an increase in the extraction temperature from 50 to 70 ℃, but after 70 ℃ caused instability of yield polysaccharides.	
	• Extraction time: 1 to 3 h	• The yield of polysaccharides increases with time but begins to decrease when a certain level is reached.	