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Tannins: Extraction from Plants

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Abstract

The chapter presents mainly on different extraction methods of tannin. Some technical means required for effective extraction are also presented, for example, collection and treatment of plant and drying and storage of plant. Opportunity and challenges in application of extraction methods are also exhibited in the chapter.

Keywords: extraction, sea plant, tannin, terrestrial plant

1. Introduction

Tannins are high molecular weight phenolic compounds commonly found in plants with molecular weights ranging from 500 to over 3000 Da and up to 20,000 Da. Chemical structure of tannin is very diverse and different. More than 8000 different tannins have been detected. Tannins have been found in both free and bound forms in plant cells. Terrestrial plant tannins can be divided into four major groups: gallotannins, ellagitannins, proanthocyanidins (condensed tannins), and complex tannins. Sea plant tannins have been described as "phlorotannins," including oligomers or polymers of phloroglucinol. Tannin content can range from 0.2 to 25% DW [1], depending on plant species, harvest time, habitat of plants, and extraction method.

Many various bioactivities of tannins have also demonstrated including antioxidant, antibacterial [2], antifungal [3], antitumor, etc. The number of hydroxyl radicals and aromatic rings is important parameter determining in bioactivity of tannins. Ortho-dihydroxyl groups of tannin are important feature for chelate metal ions. High degree of polymerization and molecular weight play an important role for antioxidant activity of tannins. Tannins can bind tightly to protein through hydrogen bonding between the phenolic groups of the tannins and the -NH groups of peptides. These hydrogen bonds cannot be broken down by digestive enzymes or the attack of microorganisms [4].

Currently, tannins have been applied into many different fields including medicine, food, beverage, manufacture of ink and adhesives, dye and tanning industry, plastic resins, water purification, and surface coatings. Their applicability depends on its concentration, as a complexing agent or a precipitating agent [5].

From the above problems, it is necessary to extract and use tannins effectively. Therefore, this chapter focuses on extraction methods of tannins including maceration, microwave, ultrasound, enzyme, decoction, irradiated radiation, and gamma and points out some issues related to extraction efficiency of tannins, for example,

harvesting, handling, and storing materials. Opportunity and challenge of tannin extraction are also presented in the chapter.

2. Harvest and treatment of sample

2.1 Sea plants

Sea plants including seagrass, seaweeds, and mangroves contain a large amount of active tannins. The only way of seagrass collection is to dive into the sea. The harvest of seaweed has two ways: the first way, diving into the sea to collect seaweed; the second way, using machines for the harvest. Mangroves can only use an equipment for the harvest. Marine plant harvest should be selected to avoid the destruction of marine vegetation. Their physiological respiration ability is very strong, and they contain large amount of salts causing quick decay of sea plants immediately after the harvest. Hence, sea plants should be washed with salt water, then with fresh water moving salts. Sea plants are dried until 19% humidity by using freeze drying, infrared-freeze drying [6], microwave-vacuum drying, assisted microwave-vacuum drying [7], or hot air after cleaning seaweed, and infrared-freeze drying is usefully evaluated [8]. Infrared radiation power intensity of $5\frac{kW}{m^2}$ is suitable.

2.2 Terrestrial plants

Terrestrial plants are more diverse than sea plants, so the method of harvesting depends on the type of plant. The structure of terrestrial plants is more stable than marine plants, and they also do not contain salts. They still have a biochemical respiration. Therefore, they should also be dried by the above methods immediately after harvest. After drying, they also need to be grinded like marine plants for study and production. Terrestrial plants and marine plants should be stored in small plastic bags under refrigeration, the thing help longer storage time.

3. Role and position of tannins in plant cells

3.1 Sea plants

Tannins (phlorotannins) in sea plants exist in integral structural of cell walls. They directly participate in the structure of cells and bind to alginate, protein, laminarin, and fucoidan. Tannins also play an important role in the formation of the zygote's cell wall [9]. They have secondary ecological roles such as against UV radiation and grazing [10]. They possess metal sequestration capacity and algicidal effectiveness [11].

3.2 Terrestrial plants

Tannins have an important role in terrestrial plants, for instance, against microbial pathogens, harmful insects, and mammalian herbivores. They help in plant growth via binding to protein. They are involved in the cell structure of plants [12]. In all vacuoles and surface wax of plants, chloroplast-derived organelle and the tannosome produce tannins. They often exist in organelles where they are less affected by the protein precipitation. In Japanese persimmon fruits, the accumulation of tannin occurs in the vacuole of the tannin cells [12, 13], and tannin/tannin-less vacuoles are found in *Mimosa pudica*. Tannins are not accumulated in the vacuoles of sensitive plants [14].

4. Extraction methods

4.1 Method of maceration

Maceration is one of the techniques used for tannin extraction from medicinal plants. Maceration is the simplest technique of extraction where the plant powder is placed in a closed vessel and soaked with the corresponding amount of solvent for a specified period of time until the tannins are dissolved in the solvent. In the first stage of the maceration, osmose occurs before diffusion occurs, in later stages, osmose occurs simultaneously with diffusion. The first stage is usually very short, and it can be a few seconds or hundredth second. The first-stage time depends on the type of solvent, the extraction material, and the extraction conditions. The movement of solvents in osmotic process from outside the cell into the cell and vice versa complies with van't Hoff's law. Dissolving solutes in osmotic process cause a pressure deficit called osmotic pressure. The osmosis is calculated on the basis of thermodynamics. Therefore, the osmotic pressure in ideal solutions is determined by Eq. (1), and the equation includes an osmotic coefficient (φs) for non-ideal solutions (Eq. 2). The osmotic coefficient depends on characteristic of each solute.

$$\pi = RT\sum Cs \tag{1}$$

$$\pi\text{observed} = RT\sum \varphi sCs \tag{2}$$

where T is the temperature; R is the universal gas constant; and Cs is the molar concentration ($C = \frac{n}{V}$).

However, the actual pressure depends on the interaction between the solute and the cell membrane. Therefore, πobserved requires the reflection coefficient, σs:

$$\pi\text{observed} = RT\sum \sigma s \varphi sCs \tag{3}$$

The osmose stops only when thermodynamic equilibrium occurs. It minimizes Gibbs free energy, $\Delta G = 0$, and temperature and pressure of solvent are fixed. Gibbs free energy is calculated by following equation:

$$G = E + \rho V - TS \tag{4}$$

where E is the energy; ρ is the pressure; V is the volume; S is the entropy ($S = k_B \ln \Omega$) inside the Boltzmann constant (kB); and number of configurations ($\ln \Omega$).

In osmotic process, osmotic pressure and hydrostatic pressure appear together playing an important role in fluid flow across the membrane. Characteristic parameters such as the permeability, the hydraulic conductivity, and the reflection coefficient reflect passive material transfer across cell membranes. Osmolarity is a concentration measure including freezing point depression, vapor pressure depression, and boiling point elevation. The diffusion in process of extraction depends on Fick's laws and Maxwell-Stefan diffusion. Therefore, the extraction yield and diffusion of tannin depend on material size, time and temperature of extraction, type of solvent, stirring, and size of tannin. Usually, the material size is optimal from 30 to 40 mesh [15]; the smaller material size creates difficulty during filtration. The material size depends on chemical characters of material and extraction method. In microwave-assisted method, material size larger than 50 meshes is suitable for the extraction and the filtration [16]. A modern mathematical exhibits Fick's first law and Fick's second law of diffusion as follow:

$$Fick's\ first\ \text{law}\ N_i = -D_i \nabla c_i \tag{5}$$

$$Fick's\ second\ \text{law}\ \frac{\partial c_i}{\partial t} = D_i \nabla^2 c_i \tag{6}$$

where N_i is the molar flux of i (mol $\frac{m^2}{s}$); D_i is the diffusion coefficient of i ($\frac{m^2}{s}$); and c_i is the concentration of i ($\frac{mol}{m^3}$).

Fick's second law exhibits the chemical species concentration being the dependent variable and diffusion of each chemical species occurring independently. When modeling diffusion of tannins, all diffusion coefficients are assumed equal and independent of temperature, pressure, etc. Fick's second law also exhibits a relation between the elapsed time and the square of diffuse distance. Fick's law is suitable for the solutions of one chemical species. Maxwell-Stefan diffusion describes good for multi-component diffusion. In Maxwell-Stefan diffusion, the species mole or mass fractions (xi and ωi, respectively) are dependent variable, and the mass flux of one chemical species is related to the concentration gradients of all chemical species. Parameterizing the diffusion rate of n components in a solution needs $n(n-1)/2$ independent coefficients. Total diffusion is equal to the total of molecular diffusion and convection term. Convection term, eddy diffusion, and molar flux due to convection are equal to each other [17, 18]. Convection term is calculated by the following equation:

$$\text{Convection term} = \text{concentration} \times \text{mass transfer velocity} = CiV \tag{7}$$

where Ci is the concentration of i ($\frac{kmol}{m^3}$) and V is the volume (m^3).

$$\text{Mass transfer velocity} = \frac{\text{mass flux}}{\text{concentration}} = \frac{N_i + N_j}{C_T} \frac{\frac{kmol}{m^2.s}}{\frac{kmol}{m^3}}; \frac{m}{s} \tag{8}$$

Thus, writing total diffusion equation for solution is

$$\text{Total diffusion} = Ni = Ji + CiV \tag{9}$$

$$N_i = -D_{ij} \frac{dC_i}{dz} + \frac{C_i}{C_T}(N_i + N_j) \tag{10}$$

where Ji is the molecular diffusion flux of i, ($\frac{kmol}{m^2.s}$); DAB is the diffusivity or diffusion coefficient for i in j, ($\frac{m^2}{s}$); and z is the distance of transfer (m).

The diffusion finishes only when mass balance of tannins between inside and outside the cell occurs. The stirring in the extraction process helps to increase the diffusion of tannins into the extract. When the mass balance of tannins occurs, new solvent is replaced to increase the efficiency of tannin extraction.

The technique is considered as a simply and low-cost way to extract tannins from medicinal plants. The technique is also a simple and popular choice for researchers because of non-complicated utensil and equipment. However, the duration of extraction time is long, the ratio of solvent to material is much, the extraction efficiency is not high, the volume of extraction tanks and the factory area are wide, compared to different techniques. The yield of tannin extraction is different when the techniques are different [19]. The yield of tannin extraction increases when hydrogen bonds between tannins and proteins are broken [20], or interactions between tannins and proteins during extraction are not formed [21, 22].

The yield of tannin extraction depends on many factors, for example, type of solvent (Snyder's solvent polarity index, pK_a, pK_b), plant species, temperature and time during the extracting process, and pressure in vessel. In the maceration, temperature of extraction can be hot, cold, or warm due to tannin characters and sample. In cold extraction, it requires thermolabile compounds. The hot extraction recommended for high temperature leading to decomposition proteins and cell structure during heating. When tannin extraction from *Galium tunetanum* Poiret, the experiment showed acetone being a better solvent, compared to ethanol (30%) [19], Na_2SO_3 solutions, NaOH solution, etc. [23]. When extracting tannins from Moroccan *Acacia mollissima* barks, methanol is evaluated being the best solvent at room temperature, and ethanol exhibits as the best solvent at 60°C [24]. Less tannin is extracted with aqueous or acidic methanol than with aqueous acetone [25]. For fresh barks of *Acacia mangium*, 50% acetone brings high yield of tannin extract [23]. The choice of the solvent types and the extraction temperature depends on the properties of the tannin in each plants. For extracting tannins from seaweed, ethanol is a best solvent because of high purified tannins and good activity. Temperature of extraction, type of solvent, and time of extraction are related to each other, and the relation exhibits clearly in **Table 1**.

Condensed tannins from grape pomace were extracted at 10°C for 120 min. The compound was then cooled, washed, and filtered. Adjusting the pH of the liquid

Solution	Condition of extraction	Reference
Ethanol	10 g of powdered plants in 100 mL of 30% ethanol at 60°C for 2 h.	[26]
Acetone	20 g of powdered plants in 200 mL of acetone for 24 h.	[27]
50% acetone	5 g of materials in 100 ml at room temperature for 24 h.	[23]
Water		
50% methanol	5 g of materials in 100 ml at room temperature for 24 h.	
Water	5 g of materials in 100 ml at 100°C for 40 min.	
Water	5 g of materials in 100 ml for 40 min, in autoclave	
2% Na_2SO_3		
4% Na_2SO_3		
6% Na_2SO_3		
0.04% NaOH solution	5 g of materials in 100 ml at 100°C for 40 min, in water-bath	
50% methanol	5 g of materials in 100 ml at 100°C for 4 h, reflux method	
50% methanol + Na_2SO_3 2%		
50% acetone		
50% acetone + Na_2SO_3 2%		
Water	Solvent and mangrove fruit ratio of 5:1 (w/w), 80°C for 60 min.	[28]
NaOH 5%	Solid-to-liquid ratio of 1:10 (w/v) at 10°C during 120 min	[29]
Water	10 g of sample in 200 ml at room temperature for 3 days	[30]
Ethanol		
50% (v/v) ethanol		

Table 1.
The conditions of tannin extraction in some literatures.

phase continuously, it reaches 1.5 by using dilute HCl for tannin precipitation. Finally, precipitated tannins were obtained at 8000 rpm for 15 minutes by centrifugation. Yield of extraction is higher than treatment of grape pomace by a solution of hydroxide sodium 5% (w/w) [29]. Maceration is similar to infusion method by which the materials are soaked in cold or boiling water for a short time [31].

4.2 Method of decoction

The decoction is similar to maceration method. However, the mixture is continuously heated at 100°C during decoction. Reaction kinetics due to the heat is stronger than the method of maceration. For extracting condensed tannins, the decoction is more effective than the maceration. In these methods, condensed tannin concentration depends on polarity of the extraction solvent. The mixture is then cooled to room temperature and the filtrate collection. Aqueous is a good solvent for the extraction of condensed tannins by decoction and maceration [32]. The decoction is very suitable for non-destroyed substances by the heat [31]. In addition to diffusion pressure and osmosis pressure, tannins are also separated from the material by the energy supplied from the thermal energy of the solvent and the affinity of the solvent. The thermal energy of the solvent separates tannins from the cells through the action on the bonds between tannins and cells. The affinity of the solvent will compete and pull tannins out of the material.

The method requires easy and cheap equipment; it is suitable for all production scales. However, the method consumes more energy than the maceration, and it is not suitable for the extraction of heat sensitive tannins [31].

4.3 Pressurized water extraction

The method is similar to infusion, hydrodistillation, decoction, and maceration. However, high pressure from 100 to 150 bar is used in the method, the thing is not found in different methods. Extraction temperature can be range from 60 to 100°C for 30 min static extraction. The solvent of water is commonly used in the method [33], and the other method includes subcritical water extraction, super-heated water extraction, pressurized liquid extraction, and accelerated solvent extraction [34–36]. Pressurized water extraction is divided into static pressurized water extraction and dynamic pressurized water extraction. The residence time of tannins in dynamic pressurized water extraction is shorter than in static pressurized water extraction, and tannins are less degraded in dynamic pressurized water extraction. Hence, the extraction efficiency and the degradation of tannins depend on the extraction time [37]. The distribution ratio of tannins into the water plays an important role in the efficiency of tannin extraction. Dynamic pressurized water extraction is a higher investment, which is more difficult to use than static pressurized water extraction [38]. The temperature of extraction is often from the atmospheric boiling point of water (100°C/273 K, 0.1 MPa) to the critical point of water (374°C/647 K, 22.1 MPa) [38].

4.4 Ultrasound method

The method of ultrasound is ultrasonic-assisted maceration method commonly used for the extraction of tannins from plants, microalgae, and seaweeds, for instance, condensed tannins, hydrolysable tannins, and valonea tannins. In the method, ultrasonic power, temperature, and time of ultrasound are the factors that strongly influence the yield of tannin extraction. Obtained tannins by

Extraction method	The solvents				
	Acetone	96% ethanol	Ethyl acetate	Chloroform	n-Hexane
Ultrasound	1.618	2.112	0.277	0.222	0.058
Maceration	3.104	4.102	0.527	0.482	0.133
Soxhlet	2.213	3.715	0.283	0.261	0.095

Table 2.
Extracted tannin content from brown algae by the solvents and methods of difference.

ultrasonic-assisted maceration method are 17.6% higher than traditional methods [39]. In the method, the efficiency also depends on sound waves [40], which produce the mechanical vibrations in the solid causing implosion of cavitation bubbles and formed sheer forces. Thus, cell structure of plant is destroyed, and the yield of tannin extraction is highly increased. The acoustic waves are often most commonly measured more than >20 kHz [40, 41]. Ultrasound-assisted extraction is the technology of low investment and high efficiency for the extraction of tannins [42]. Ultrasound–assisted extraction method leads to efficient improvement of mass transfer and tannin extraction yield, reducing consumption of time and solvent, compared to the conventional method [43]. Ultrasound-assisted extraction method is environmentally friendly method, less risk of chemistry and physics, and less impact on molecular structural properties of tannins in plants [44, 45].

In the last decade, numerous publications on the use of ultrasound in active tannin extraction have been found. Ultrasonic frequency is commonly used being 40 kHz, ultrasonic time is about 30 min, and ultrasonic temperature can be a room temperature or warm temperature (55°C) [46, 47]. Analysis results of tannin content in brown algae commonly growing in Vietnam showed that the yield of tannin extraction by maceration is better than ultrasound and soxhlet. The thing is in contrast to tannins in terrestrial plants, and the expression of tannins in seaweed is more easily destroyed than tannins in terrestrial plants. Ninety-six percent ethanol is determined being the best solvent for tannin extraction from brown algae (**Table 2**), and the thing showing tannins in brown algae has a strong polar.

Some publications showed that ultrasonic frequency of 20 kHz and ultrasonic power ranging from 30 to 200 W are used for the extraction of tannins [39].

The dissolution rate of tannins from the plant material can be presented by Eq. (11) [48].

$$\frac{dC_t}{dt} = k(C_s - C_t)^2 \tag{11}$$

where Ct is the concentration of tannins in the extract at a time t (min), (mg $\frac{GAE}{L}$); Cs is the saturated concentration of tannins in the extracts, (mg $\frac{GAE}{L}$); and k is the second-order extraction rate constant, $\left(\frac{\frac{L}{g}}{\min}\right)$.

For determining kinetic parameters, some assumptions are given, t = 0 to t, Ct = 0 to Ct, the equation is written in the form of Eq. (12).

$$C_t = \frac{C_s^2.k.t}{1 + C_s.k.t} \tag{12}$$

where k is calculated according to the Arrhenius law

$$k = k_0 \exp\left(\frac{-E_a}{RT}\right) \tag{13}$$

where E_a is the value of the activation energy; R is the gas constant ($8.314 \frac{J}{mol}$ K); and T is the temperature of the extraction (K).

Ultrasonic-assisted extraction is also combined with ionic liquid improving extraction efficiency [49].

4.5 Microwave-assisted extraction

The microwave-assisted extraction is a good choice for the extraction of tannins using microwave (electromagnetic radiations in the frequency range from 300 MHz to 300 GHz) energy [50]. The heating mechanism of microwave is very special [51]. Ionic conduction and dipole rotation cause the transformation of the microwave energy to heat through interacting with polar molecules [50]. The microwave supplies the high temperature (100–150°C) in the extraction process [52]. The yield of tannin extraction strongly depends on the penetration depth of microwaves into plants and the power of microwaves. Inside the dielectric constant, the moisture content and temperature of plants, and the frequency of the electrical field decide the penetration of microwave depth. The water in plants absorbs microwave energy to reach the superheated state and cell structure disruption. The thing is to help to increase the diffusion of tannins into the extract. The solvents such as water, ethanol, and methanol strongly absorb microwaves energy, and their dielectric constant is high, compared to toluene or hexane. Toluene and hexane maintain the surrounding extraction solvent in the cold state because of their dielectric constant. Water, ethanol, and methanol are advised for the extraction of tannins, because tannins are very soluble in them [53]. Microwave-assisted maceration is a simple, time-saving method, less cost of solvent, and energy. The microwave technique brings the higher efficiency of tannin extraction than ultrasound method [54, 55]. The yield of tannin extraction in microwave method is 1.25 times more than maceration method [24]. The efficiency of tannin extraction is arranged in descending order according to the following methods: microwave, maceration, and infusion. Microwave-assisted extraction is less efficiency for non-polar tannins and non-polar solvents [56].

4.6 Ionic liquid-based microwave-assisted extraction

Ionic liquid-based microwave-assisted extraction is a high efficient combination method in tannin extraction from plants. Ionic liquid aqueous solution-to-material ratio of 20:1 is suitable in the method. Currently, there are numerous ionic liquids, for example, [Emim]Br, [Bmim]Br, [Hmim]Br, [Omim]Br, [Dmim]Br, [Bmim]Cl, [Bmim]BF4, [Bmim]NO3, and [Bmim]OH, where Emim is 1-ethyl-3-methylimidazolium; Bmim is 1-butyl-3-methylimidazolium; Hmim is 1-hexyl-3-methylimidazolium; Omim is 1-octyl-3-methylimidazolium; and Dmim is 1-decyl-3-methylimidazolium.

In the method, the materials are soaked in ionic liquid for 3 h before microwave-assisted extraction. Extraction time of tannins by microwave is about 10 min under microwave power of 230 W. A 1.25 M sodium chloride and 80% ethanol are used as the solvent in ionic liquid-based microwave-assisted extraction [57]. This is a friendly method and low energy consumption but high cost.

4.7 Infrared-assisted extraction

Infrared-assisted extraction is a maceration using an infrared lamp to supply the heat, and the penetration of infrared into the material is higher than other

method [58]. In conventional methods, the solvent is heated before heating the material. In infrared method, the material is directly heated without heating the solvent [59]. The effectiveness of the infrared method depends on the absorption characteristics of the extracting solvent, the wavelength of the infrared heater, and the distance between the lamp and the material. Electromagnetic waves excite tannins of material in the modes of twisting, stretching, and bending, increasing the efficiency of tannin extraction [59, 60]. Infrared method exhibits a higher extraction yield than microwave-assisted extraction and ultrasound extraction [41]. Infrared-assisted extraction is low cost, but extraction efficiency of tannins from plants is high, and the time of infrared extraction is about 30 min [60].

4.8 Soxhlet extraction

Extraction method and solvent play an important role in the extraction of tannins. Soxhlet extraction is a good method when compared to cold maceration. The effectiveness of soxhlet extraction is mainly based on the evaporation temperature and polarity index of the solvent, and some characters of solvents are presented in **Table 3**. The suitable temperature of extraction improves the diffusion ratio of tannins into the solvent and the circulation of the solvent. Fifty percent ethanol gives the highest extract yield of tannins from herbal plants [30]. Soxhlet extraction with water content is also evaluated as the best method for gallic and ellagic acid extractions. Solvent polarity, solvent-to-solid ratio, and contact time significantly affect the yield of tannin extraction [33]. The solvents such as water or ethanol-water mixtures are used for the extraction of the active hydrolysable tannins (gallic acid and ellagic acid). Extract yield of tannins corresponded to 27.1, 26.4, 26.2, 22.5, 14.6, and 11.6 (% g/g sample) when using extraction solvent of ethanol:water (20%:80%), ethanol:water (30%:70%), water, ethanol:water (50%:50%), methanol, and ethanol, respectively, in the method [33]. Extract yield of tannins is the lowest with n-hexane. Synder's solvent polarity index almost causes high extract yield of tannins. Synder's solvent polarity index is calculated according to the following equation [61]:

$$\text{Synder's solvent polarity index} = \left(\frac{I_A}{100}.P_A\right) + \left(\frac{I_B}{100}.P_B\right) \qquad (14)$$

Extraction solvent	Synder's solvent polarity index	Boiling point (°C)	Extraction solvent	Synder's solvent polarity index	Boiling point (°C)
n-Hexane	0.1	69	70% acetone, 30% water	6.5	84
Dichloromethane	3.4	40	70% ethanol, 30% water	8.2	90
Chloroform	4.1	61	50% ethanol, 50% water	7.9	94
Acetone	5.4	56	30% ethanol, 70% water	7.1	97
Ethanol	5.2	78	20% ethanol, 80% water	6.3	98
Methanol	6.6	65	Water	9.0	100

Table 3.
Synder's solvent polarity index and boiling point of some solvents.

where I_A and I_B are the polarity index of solvents A and B, respectively; P_A and P_B are the percentage of solvents A and B, respectively.

However, soxhlet method needs long duration and high amount of solvent, hence the loss efficiency of economic and the environmental problems. When the evaporation temperature of the solvents is high, thermal destruction of tannins can happen [53]. Extraction time is about 3 h or 6 h due to the materials [33].

4.9 Supercritical fluid extraction

The supercritical fluid extraction is a modern and environment friendly method, which maintains the chemical and biological characters of tannins. The supercritical solvents have an important role in the efficiency of tannin extraction, and **Table 4** presents some properties of useful solvents for tannin extraction. The supercritical solvents are used as extraction solvent in the method including carbon dioxide, hexane, pentane, butane, nitrous oxide, sulfur hexafluoride, and fluorinated hydrocarbons. Supercritical carbon dioxide is often the choice because of its low critical pressure (100 and 450 bar) [62]. However, the selectivity of CO_2 is not good. For improving the problems, a co-solvent or modifier is combined with CO_2 in tannin extraction [63]. Water and ethanol-water cosolvents exhibit a good result in the separation of the less polar compounds and the hydrolysable tannins [53]. The diffusion coefficient of the supercritical fluid is higher than a liquid solvent, but the viscosity and the surface tension of the supercritical fluid are lower than a liquid solvent. Hence, the diffusion and mass transfer of tannins are better when using the supercritical fluid. The solvation power of supercritical fluid can be changed via the change of temperature and/or pressure [53, 56].

From the parameters in **Table 4** and previous claims, it is easy to see that the higher the viscosity of the solvent is, the greater the solubility of tannins is.

4.10 Enzyme-assisted extraction

Enzyme-assisted extraction is one of the environmentally friendly methods using an enzyme or complex enzymes to improve bioactive extraction yield via the disruption of the material cell. This method is combined with various methods, but high temperature is not used for the extraction of tannins because protein is precipitated under high temperature. Extraction time or time of enzyme treatment is sufficient and not long because a long time leads to release tannins and the formation of protein-tannin complexes. Of course, not all tannins precipitate all proteins, this precipitation is selective. The tannin-protein complexes are only formed when

Solvent	The moment of dipole (Debye)	The constant of the dielectric	Cohesive energy density ($J \frac{mol}{mL}$)	Viscosity (mPa)	The tension of surface ($\frac{Cal}{mol}$)
n-Hexane	0.00	1.88	200.76	0.30	25.75
Acetone	2.88	20.49	362.07	0.31	33.77
Chloroform	1.04	4.71	332.00	0.54	38.39
Ethanol	1.69	24.85	618.87	1.07	31.62
Methanol	1.70	32.61	808.26	0.54	31.77
Water	1.87	78.36	2095.93	0.89	104.70

Table 4.
Some properties of solvents used for tannin extraction [64].

proteins and tannins have high molecular weight, an open flexible structure, hydrophobic proteins of proline richness [65, 66]. The linkages between tannins and proteins are unstable. The linkages between proteins and tannins are formed from phenolic groups of tannins and the carbonyl groups of peptides [65]. Four types of bonds between proteins and tannins are suggested in **Table 5** [67].

For enhancing the extraction effectiveness, the treatment of the material cell by the enzyme complex should be used prior to tannin extraction. Enzymatic pretreatment achieves a reduction in the extraction time and energy consumption [68]. Enzymes complexes weaken or break down the linkages between tannins and cell wall [69–71]. In this way, the partial or overall degradation of material cells allows leasing tannins from plants [72, 73]. The cell-wall degrading enzymes can be used for tannin extraction including Celluclast® 1.5 L, Pectinex® Ultra, Novoferm®, and hemicellulases. The treatment of seaweed by three different enzymes shows that the efficiency of the cell membrane of seaweed is significantly different. Viscozyme L enzyme destroys the seaweed cells better than termamyl enzyme (**Figures 1** and **2**), and eroding ability of seaweed cell membrane of cellulase enzyme at 60°C is better at 50°C (**Figures 3** and **4**).

Cellulase enzyme usually exhibits high efficiency in tannin extraction from seaweed at other temperatures, compared to other enzymes (**Figure 5**). Enzyme-assisted extraction permits the extraction of tannins with high stability and high activity. This is fully feasible because catalyze reactions and regioselectivity of enzymes can take place in aqueous medium under mild conditions [74]. Disrupting the structural integrity of the material cells by enzymes is the easy method and low solvent consumption. However, a clear understanding of the catalytic property, the material structure, the mode of action, and the enzyme conditions is

Types of bonds	Interactions	Control factor
Hydrogen bonds	The oxygen of amide groups in the peptide bonds of proteins and the hydroxyl radicals of the tannins	Reversible and dependent on pH
Hydrophobic interactions	The hydrophobic regions of the proteins and the aromatic rings of the tannins	Reversible and dependent of pH
Ionic bonds	The cationic sites of the proteins and the phenolate anion	
Covalent bonding	The oxidation of polyphenols to quinones and their subsequent condensation	Irreversible

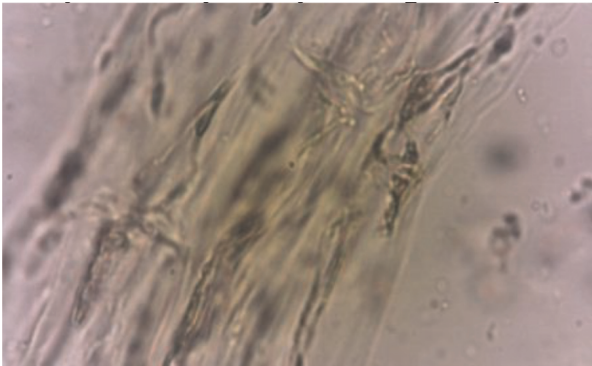**Table 5.**
Some types of bonds between proteins and tannins.

Figure 1.
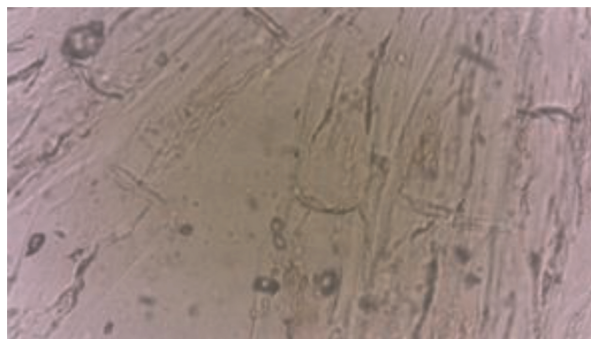*A part of algae stem Sargassum olygocystum after being treated at 40°C with 0.3% enzyme Termamyl.*

Figure 2.
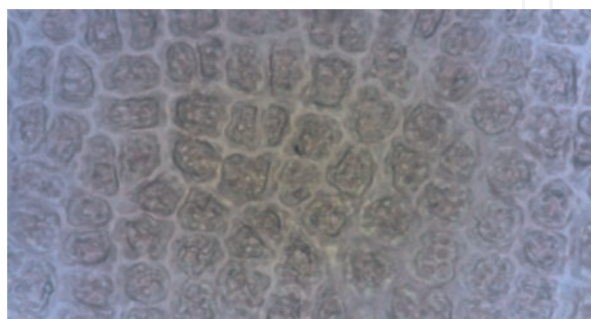*A part of algae stem Sargassum olygocystum after being treated at 40°C with 0.3% enzyme Viscozyme L.*

Figure 3.
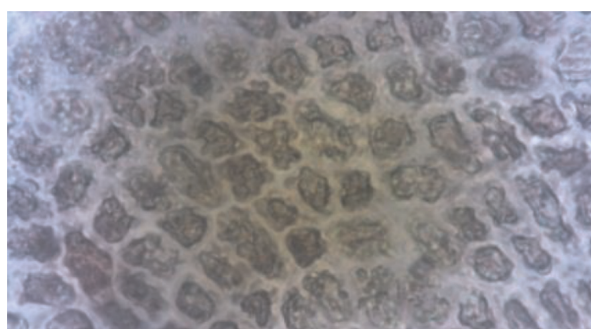*A part of algae leave Sargassum olygocystum after being treated at 50°C with 0.3% enzyme cellulase.*

Figure 4.
A part of algae leave Sargassum olygocystum after being treated at 60°C with 0.3% enzyme cellulase.

necessary [68]. The effectiveness of tannin extraction mainly depends on ionic strength [75], ions in solution [76], and characters of proteins and tannins. Some problems on technology need to consider before large production, for example, the price of enzymes, hydrolyze capacity, and operating conditions of enzymes [68].

4.11 Gamma-assisted extraction

Gamma-assisted extraction is a new method using Co-60 gamma radiation for tannin extraction. Co-60 gamma radiation increases the effectiveness of the tannins from *Anacardium occidentale* [77]. However, this method still needs more study to apply, and expanding ability in large scale also entails many legal issues. For tannin extraction, materials can be directly irradiated by gamma radiation, materials are then soaked in specific solvent, or the mixture of materials and solvents is directly irradiated by gamma radiation. Radiation dose of 5–25 kGy is concordant with tannin extraction from brown algae.
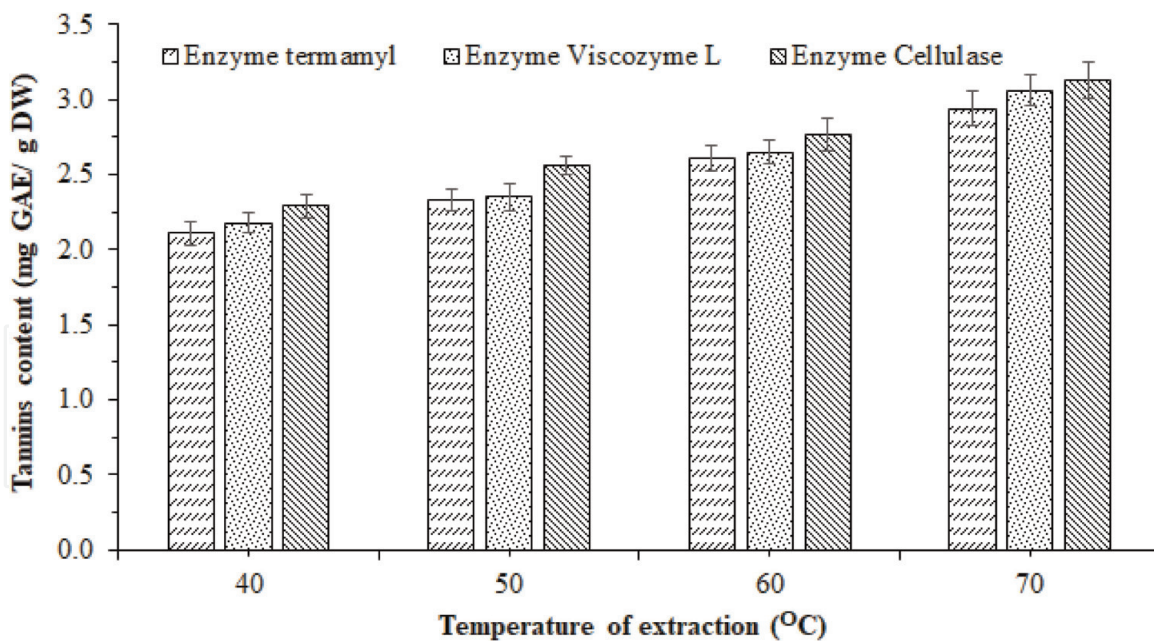
Figure 5.
Extracted tannin content from brown algae in different conditions of enzyme and temperature.

5. Opportunity and challenge of tannin extraction

Tannins commonly exist in the nature, and they are bioactive diversity such as antioxidant, antifungal, anti-virus, anti-inflammatory, and anti-parasitic. Tannins are used in numerous fields, for example, feed of ruminants, monogastric animals, etc. Each method of tannin extraction has difficult and easy points, for example, high investment, difficult operation, or popular equipment. However, all methods are based on the dynamic and static maceration. All modern techniques are supporting and improving traditional extraction methods. At the same time, the demand using tannins in the market is very huge. Therefore, opportunity of tannin extraction and commercializing tannins are great.

However, large-scale production of tannins greatly differs in comparison to small-scale production. The content, structure, and activity of tannins in plants depend on growth time of plants, habitat place of plants, species, the parts of the plants, etc. [78]. Hence, stabilizing the content, structure, and activity of tannin is difficult. Tannin content is decreased in storage time of materials. The results of the tannin activities are mainly at the laboratory level. At the same time, modern method of tannin extraction needs high investment, good manpower, and great market. There are some challenges in tannin extraction and its application into the life.

6. Conclusions

The chapter presents the position and the role of tannins in plant cell structure, the harvest and treatment of plant materials, the opportunities, and the challenges of tannin extraction from plants to apply into various fields. Specially, traditional and modern methods of tannin extraction are also discussed specifically including the extraction techniques, the advantages and the disadvantages of the method, the mechanism of the extraction process, the solutions to efficiency improvement of tannin extraction, some experimental parameters of tannin extraction, some conversion process in the extraction, mathematical model of transformation, and kinetics of extraction process.

The presented extraction methods have the advantages and the disadvantages, but they can be used effectively in tannin extraction from plants. The methods of tannin extraction should continue to be improved to bring higher efficiency.

Conflict of interest

Non conflict of interest declaration.

Abbreviations

DW	dry weight
GAE	gallic acid equivalent
g	gram
kGy	kilogray
Min	minutes
v	volume
w	weight
W	watt
M	mol

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