technologies

MDPI

Review

Phytochemicals Derived from *Catharanthus roseus* and Their Health Benefits

Hong Ngoc Thuy Pham [1,2,*], Quan Van Vuong [1], Michael C. Bowyer [1] and Christopher J. Scarlett [1,*]

1 School of Environmental and Life Sciences, Faculty of Science, University of Newcastle, Ourimbah, NSW 2258, Australia; vanquan.vuong@newcastle.edu.au (Q.V.V.); michael.bowyer@newcastle.edu.au (M.C.B.)
2 Faculty of Food Technology, Nha Trang University, No. 2 Nguyen Dinh Chieu Street, Nha Trang City 650000, Vietnam
* Correspondence: hongngocthuy.pham@uon.edu.au (H.N.T.P.); c.scarlett@newcastle.edu.au (C.J.S.)

Received: 18 November 2020; Accepted: 17 December 2020; Published: 21 December 2020

Abstract: *Catharanthus roseus* (*C. roseus*) is an important medicinal plant distributed in many countries. It has attracted increasing attention due to it being shown to possess a range of phytochemicals with various biological activities such as antioxidant, antibacterial, antifungal, antidiabetic and anticancer properties. Remarkably, vinblastine and vincristine isolated from this plant were the first plant-derived anticancer agents deployed for clinical use. Recently, new isolated indole alkaloids from this plant including catharoseumine, 14′,15′-didehydrocyclovinblastine, 17-deacetoxycyclovinblastine and 17-deacetoxyvinamidine effectively inhibited human cancer cell lines in vitro. Moreover, vindoline, vindolidine, vindolicine and vindolinine isolated from *C. roseus* leaf exhibited in vitro antidiabetic property. These findings strongly indicate that this plant is still a promising source of bioactive compounds, which should be further investigated. This paper provides an overview of the traditional use and phytochemical profiles of *C. roseus*, and summarises updated techniques of the preparation of dried material, extraction and isolation of bioactive compounds from this plant. In addition, purported health benefits of the extracts and bioactive compounds derived from this plant were also addressed to support their potential as therapeutic agents.

Keywords: *Catharanthus roseus*; bioactive compounds; phytochemicals; health benefits

1. Introduction

Natural resources including herbal plants, which contain a large variety of phytochemicals promising as traditional medicine to treat chronic and infectious diseases, have been considered as safe and effective alternatives with fewer side effects compared to synthetic agents [1]. Amongst the plethora of medicinal plants identified, *Catharanthus roseus* (L.) G. Don (*C. roseus*) has been widely used to treat various diseases in many countries. The hot water extract of the dried *C. roseus* leaf has been used for the treatment of diabetes in Jamaica, Kenya and the West Indies or the hot water extract of the dried plant has been taken orally as complementary and alternative therapies for various types of cancers, heart disease and leishmaniasis in Peru [2]. More scientific evidence has proved the potential health benefits of individual phytochemicals extracted from this plant. Of note, vinblastine and vincristine from *C. roseus*, and their synthetic analogues, have been used in combination with other cancer chemotherapeutic drugs for treating advanced testicular cancer, breast and lung cancers [3].

The drying and extraction process are crucial steps prior to isolation and purification of bioactive compounds from plant material. Drying aims to remove moisture content and reduce water activity in plant material, thus inactivating the enzymes responsible for degrading many bioactive compounds,

decreasing the rate of microbial growth and reducing the costs of transportation and preservation. However, the drying treatment has been found to have a significant effect on the retention of bioactive compounds and antioxidant capacity of plant material [4–7]. Therefore, selecting a suitable drying method is very important to maintain the high yield of bioactive compounds and antioxidant power within plant material; however, the economic aspect and the accessibility of drying equipment should also be considered. Similarly, the extraction process is also a key step to obtain bioactive compounds from plant materials. Extraction techniques have been reported to significantly affect the extraction efficiency and stability of bioactive compounds [8–11]. Hence, this paper provides a comprehensive review of different techniques of drying, extracting and isolating bioactive compounds from the target plant material, *C. roseus*, and discusses its potential health benefits along with its traditional use.

2. Taxonomy and Traditional Use of *C. roseus*

C. roseus is native to Madagascar and known as the Madagascar periwinkle. It is now grown in many countries and is a common decorative, easy growing and spreading perennial herb. The *C. roseus* plant is 30–60 cm tall with young pubescent branches. Its leaves are oblong or oblanceolate, membranous, entire, obtuse or mucronate, and have short petioles (Figure 1). Inflorescence occurs at the axillary with 1–4 flowered cymes, and flowers are from white to red depending on the cultivar. The calyx tube is short, but the corolla tube is long, sparsely pubescent above and the throat is hairy within or below the stamens. The ovary is pubescent, long and the stigma is pentagonal. Its fruits are 15–25 mm long and have two follicles [12].

Figure 1. *Catharanthus roseus* (L.) G. Don (Patricia White cultivar) (Pham, 2014).

This plant has been used as a traditional medicine in many countries (Table 1). The dried leaf or entire plant is boiled with water and then the extract is taken orally to treat diabetes in Northeast India, the Cook Islands, Australia, England, Thailand, Natal, Mozambique, Philippines, Dominican Republic, Jamaica, Northern Europe and Vietnam [13–17]. The aqueous extract of the leaf or the whole plant is also used by Cook Island and Vietnamese people [13,17], and Kenyans as complementary and alternative therapies for various types of cancer including throat, stomach and oesophageal cancers [18]. The people in the Kancheepuram District of Tamil Nadu, India mix the powder of *C. roseus* whole plant with cow's milk, which is taken orally to treat diabetes [19]. *C. roseus* root is dried, ground and decocted for curing urogenital infections in the Venda region, South Africa [20], gonorrhoea in Limpopo Province, South Africa [21] and stomach ache in the Mutirikwi area of Zimbabwe [22].

Table 1. Traditional use of *C. roseus* in some countries.

Part Used	Disease	Preparation	Mode of Administration	Country	References
Whole plant, leaf	Diabetes	The whole plant is powdered and mixed with cow's milk.	Oral intake	Kancheepuram district of Tamil Nadu, India	[19]
	Diabetes	The leaf is boiled with water.	Oral intake	Northeast India	[14]
Leaf	Diabetes mellitus	The dried leaf is decocted.	Oral intake	Northern Europe	[16]
Leaf of purple or white flowered varieties	Diabetes, hypertension and cancer	Eighteen leaves are boiled in a kettle of water. The cool solution is drunk daily.	Oral intake	Cook Island	[13]
Whole plant	Throat, stomach, oesophageal cancer	The whole plant is boiled with water. Pound	Oral intake. Usually taken together with *Sesbania sesban* whole plant. Applied topically	Kenya	[18]
Root	Urogenital infections	The root is air dried, ground and decocted.	Oral intake	Venda region, South Africa	[20]
Root	Gonorrhoea	The root is boiled for 20 min.	Oral intake	Limpopo Province, South Africa	[21]
Root	Stomach	Crushed roots are mixed with a cup of water.	Oral intake	Mutirikwi area of Zimbabwe	[22]
Whole plant	Diabetes, hypertension, dysentery, cancer	The whole plant is boiled with water.	Oral intake	Vietnam	[17]

3. Major Bioactive Compounds Derived from *C. roseus*

A range of alkaloids (nitrogen-containing organic compounds other than amino acids, peptides, purines and derivatives, amino sugars and antibiotics) [23] have been found in *C. roseus* (Table 2). Alkaloids are broadly classified as heterocyclic or non-heterocyclic, depending on the chemical structure. Heterocyclic alkaloids are those that contain the nitrogen atom in the ring system, the size of which leads to further subclassification as pyrrole, pyrrolizidine, pyridine, piperidine, quinoline, isoquinoline, norlupinane or indole alkaloids. Non-heterocyclic alkaloids, which are also sometimes called proto-alkaloids or biological amines, are less commonly found in nature. These molecules have a nitrogen atom, which is not a part of any ring system such as ephedrine, cathinone and colchicine [23].

Table 2. Identified alkaloids in *C. roseus*.

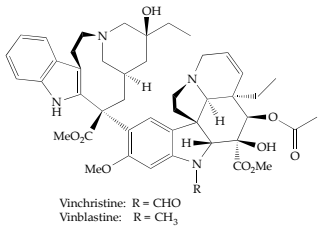
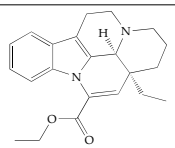
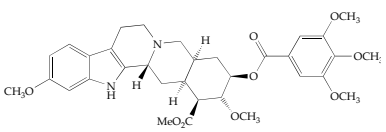
Alkaloids	Plant Part	Quantity	Chemical Structure	References
Vincristine	Leaf	0.189–0.523 mg/g DE	 Vinchristine: R = CHO Vinblastine: R = CH_3	[24]
	Stem	0.082–0.388 mg/g DE		
	Root	0.078–0.659 mg/g DE		
Vinblastine	Leaf	0.266–1.293 mg/g DE		
	Stem	0.285–1.056 mg/g DE		
	Root	0.463–1.638 mg/g DE		
Vinpocetine	Leaf	0.001–0.006 mg/g DE		[24]
	Stem	0.001–0.007 mg/g DE		
	Root	0.001–0.056 mg/g DE		
Reserpine	Leaf	0.001–0.036 mg/g DE		[24]
	Stem	0.003–0.055 mg/g DE		
	Root	0.001–0.036 mg/g DE		

Table 2. *Cont.*

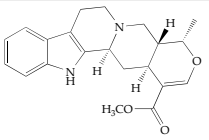
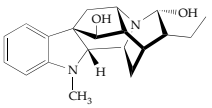
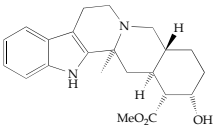
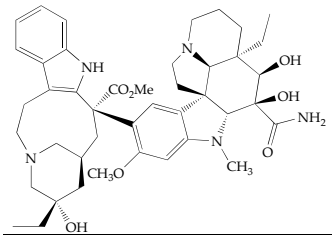
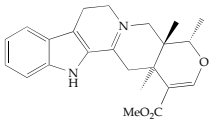
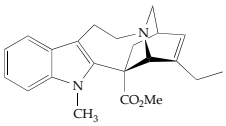
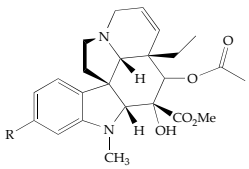
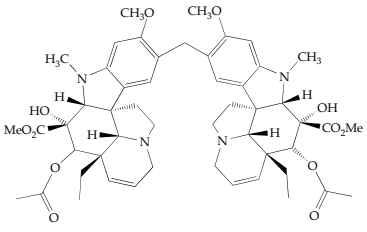
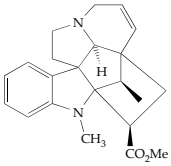
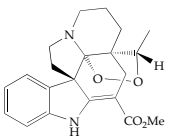
Alkaloids	Plant Part	Quantity	Chemical Structure	References
Ajmalicine	Leaf	0.165–0.970 mg/g DE		[24]
	Stem	0.162–5.487 mg/g DE		
	Root	0.124–17.675 mg/g DE		
Ajmaline	Leaf	0.016–0.067 mg/g DE		[24]
	Stem	0.025–0.085 mg/g DE		
	Root	0.036–0.140 mg/g DE		
Yohimbine	Leaf	0.139–0.539 mg/g DE		[24]
	Stem	0.185–1.572 mg/g DE		
	Root	0.316–2.433 mg/g DE		
Vindesine	Leaf	0.139–2.978 mg/g DE		[24]
	Stem	1.754–2.302 mg/g DE		
	Root	1.552–3.247 mg/g DE		
Serpentine	Leaf	2.868–22.079 mg/g DE		[24]
	Stem	11.265–50.078 mg/g DE		
	Root	4.927–49.851 mg/g DE		
Catharanthine	Leaf	0.2843 ± 0.0132 mg/g		[25,26]
Vindolidine	Leaf	0.14%	 Vindolidine: R = H Vindoline: R = OCH_3	[24,27]
Vindoline	Leaf	5.301–19.463 mg/g DE		
	Stem	0.144–3.344 mg/g DE		
	Root	0.021–9.690 mg/g DE		
Vindolicine	Leaf	0.07%		[27]
Vindolinine	Leaf	0.02%		[27]
Catharoseumine	Whole plant	0.786 mg/kg		[28]

Table 2. *Cont.*

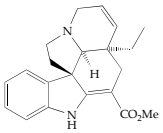
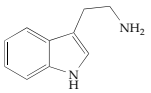
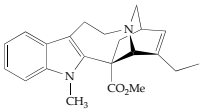
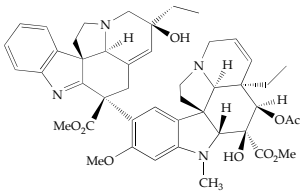
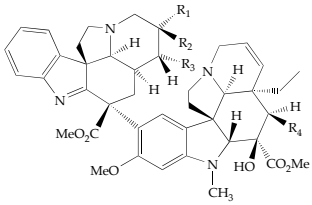
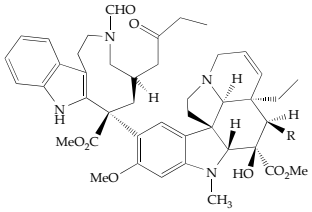
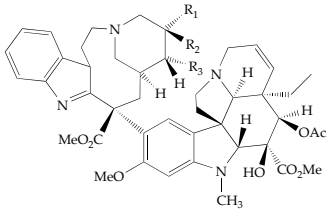
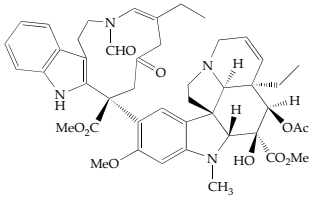
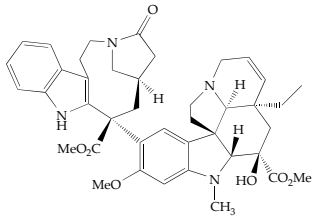
Alkaloids	Plant Part	Quantity	Chemical Structure	References
Tabersonine	Hairy root	NR		[26]
Tryptamine	Hairy root	NR		[26]
Catharanthamine	Leaf	NR		[29]
14′,15′-Didehydr ocyclovinblastine	Whole plant	0.071 mg/g DE		[30]
17-Deacetoxycy clovinblastine	Whole plant	0.089 mg/g DE	 17-Deacetoxycyclovinblastine: R_1 = Et, R_2 = OH, R_3 = R_4 = H Cycloleurosine: R_1 = R_3 = -O-, R_2 = Et, R_4 = OAc	[30]
Cycloleurosine	Whole plant	0.116 mg/g DE		
17-Deaceto xyvinamidine	Whole plant	0.107 mg/g DE	 17-Deacetoxyvinamidine: R = H Vinamidine: R = OAc	[30]
Vinamidine	Whole plant	0.071 mg/g DE		
Leurosine	Whole plant	0.134 mg/g DE	 Leurosine: R_1 = R_3 = -O-, R_2 = Et Leurosidine: R_1 = OH, R_2 = Et, R_3 = H	[30]
Leurosidine	Whole plant	0.107 mg/g DE		
Catharine	Whole plant	0.098 mg/g DE		[30]

Table 2. *Cont.*

Alkaloids	Plant Part	Quantity	Chemical Structure	References
Cathachunine	Whole plant	0.223 mg/g DE		[31]

DE: Dry extract. NR: Not reported.

Besides well-known alkaloids, new phenolic compounds were also found in the *C. roseus* leaf, stem, seed and petal (Table 3).

Table 3. Identified phenolic compounds in *C. roseus*.

Plant Parts	Phenolic Compounds	Quantity (mg/kg Dry Basis)	References
Stem	3-*O*-caffeoylquinic acid	769.9 ± 12.7	[32,33]
	4-*O*-caffeoylquinic acid	2874.6 ± 151.6	[32,33]
	5-*O*-caffeoylquinic acid	22.5 ± 1.5	[32,33]
	Quercetin-3-*O*-(2,6-di-*O*-rhamnosyl-galactoside)	190.5 ± 3.1	[32,33]
	Kaempferol-3-*O*-(2,6-di-*O*-rhamnosyl-galactoside)	190.8 ± 5.3	[32,33]
	Isorhamnetin-3-*O*-(2,6-di-*O*-rhamnosyl galactoside)	78.6 ± 3.9	[32,33]
Leaf	3-*O*-caffeoylquinic acid	2971.6 ± 15.6	[32,33]
	Kaempferol-3-*O*-(2,6-di-*O*-rhamnosyl-galactoside)-7-*O*-hexoside	52.7 ± 1.0	[32,33]
	4-*O*-caffeoylquinic acid	5156.8 ± 137.2	[32,33]
	5-*O*-caffeoylquinic acid	187.7 ± 0.5	[32,33]
	Quercetin-3-*O*-(2,6-di-*O*-rhamnosyl-galactoside)	310.9 ± 5.0	[32,33]
	Kaempferol-3-*O*-(2,6-di-*O*-rhamnosyl-galactoside)	8.5 ± 5.3	[32,33]
Seeds	Kaempferol-3-*O*-(2,6-di-*O*-rhamnosyl-galactoside)-7-*O*-hexoside	292.3 ± 0.3	[32,33]
	Quercetin-3-*O*-(2,6-di-*O*-rhamnosyl-galactoside)	582.7 ± 6.6	[32,33]
	Kaempferol-3-*O*-(2,6-di-*O*-rhamnosyl-galactoside)	2714.2 ± 4.3	[32,33]
	Kaempferol-3-*O*-(2,6-di-*O*-rhamnosyl-glucoside)	56.6 ± 0.4	[32,33]
	Isorhamnetin-3-*O*-(2,6-di-*O*-rhamnosyl-glucoside)	354.1 ± 8.2	[32,33]
	Kaempferol-3-*O*-(6-*O*-rhamnosyl-galactoside)	112.1 ± 16.0	[32,33]
	Isorhamnetin-3-*O*-(6-*O*-rhamnosyl-glucoside)	372.0 ± 65.2	[32,33]
Petal	4-*O*-caffeoylquinic acid	11153.2 ± 126.4	[32,33]
	Quercetin-3-*O*-(2,6-di-*O*-rhamnosyl-galactoside)	1027.9 ± 7.0	[32]
	Kaempferol-3-*O*-(2,6-di-*O*-rhamnosyl-galactoside)	8120.8 ± 74.4	[32]
	Kaempferol-3-*O*-(2,6-di-*O*-rhamnosyl-glucoside)	4296.3 ± 34.4	[32]
	Kaempferol-3-*O*-(6-*O*-rhamnosyl-galactoside)	9567.2 ± 98.5	[32,33]
	Kaempferol-3-*O*-(6-*O*-rhamnosyl-glucoside)	4639.8 ± 21.9	[32,33]
	Isorhamnetin-3-*O*-(6-*O*-rhamnosyl-galactoside)	989.2 ± 33.0	[32,33]
	Isorhamnetin-3-*O*-(6-*O*-rhamnosyl-glucoside)	1330.4 ± 10.8	[32,33]

This plant has been found to possess a number of important bioactive components that greatly contribute to the herbal medicine industry; however, their amounts present in the plant are often low. Of note, the biosynthesis of plant secondary metabolites is affected by the biotic and abiotic factors. Particularly, environmental stresses have been found to stimulate the production of secondary metabolites including alkaloids because of the plant protective function. Hence, more in vitro and in vivo studies focus on increasing the amounts of these compounds through changing environmental conditions such as light, salinity, soil types and nutrients, drought and metal stress. In particular, Misra and Gupta [34] found that salinity (100 mM NaCl) stimulated indole alkaloid accumulation in the *C. roseus* leaves and roots. The amount of ajmalicine in *C. roseus* increased with the treatment of cadmium (concentration ranging from 0.05 to 0.4 mM) from 24 to 48 h and excretion into the culture medium, especially for cells at the mid-exponential growth phase [35]. The study of Binder et al. [36] indicated that UV-B light stimulated alkaloid production in *C. roseus* hairy root due to protein kinase stimulation. Additionally, biomass and alkaloid yield in cell suspension culture of *C. roseus* was

found to increase along with the increased total nitrogen and phosphate at an adequate pH of test culture medium because nitrogen is an important component of alkaloids, while phosphorus affects the synthesis of alkaloids [37]. A similar phenomenon was also found in other medicinal plants. Oueslati et al. [38] found that polyphenol content of the *Mentha pulegium* leaf irrigated with a nutrient solution containing 100 mM NaCl for 14 days increased more than 3-fold higher than that of the control. The levels of terpenes increased in the green leaves of the thyme plants in response to drought stress [39].

4. Preparation and Recovery of Bioactive Compounds from *C. roseus*

4.1. Preparation of Dried C. roseus

In previous studies, various drying methods, such as freeze drying, air drying, low-temperature drying, infrared drying or drying under the shade have been applied to prepare dried *C. roseus* for further recovery of bioactive compounds (Table 5). The results showed that drying methods significantly affected the retention of bioactive compounds in *C. roseus* material because bioactive compounds are sensitive to heat, light and oxygen [40]. Freeze drying has been reported as a prominent and effective drying method in terms of the retention of bioactive compounds, but it is costly and not typically available in some of the regions where the plant is collected and processed [5]. Among thermal drying methods, infrared drying at 35 °C was found to be suitable for saponin retention within the *C. roseus* stem and root, while vacuum drying at 50 °C was suggested for drying the leaf and the flower, which contained high levels of phenolics and flavonoids [40]. However, there is no report on the effect of drying conditions on the individual bioactive compounds present in this plant material, suggesting future studies on investigating the optimum drying conditions for recovering target compounds from *C. roseus* are required.

Table 4. Preparation and recovery of bioactive compounds from *C. roseus*.

Plant Part	Compounds	Procedures	References
Hairy root	Vindoline, ajmalicine, serpentine, and catharanthine	Freeze-drying sample, Extracting sample (200−250 mg) with 80 mL of methanol for 3 h in a Soxhlet extraction apparatus (reflux rate of 12−15 siphons/h), Evaporating and diluting with 1.5 volumes of a 5 mM $(NH_4)_2HPO_4$ solution, Fractionating using a 300-mg C18 Maxi-Clean cartridge, then eluting successively with 4 mL of mixture of MeOH:5 mM $(NH_4)_2HPO_4$ (60:40, 95:5, and 100:0, *v*/*v*).	[41]
Leaf	Vindoline, catharanthine, vincristine and vinblastine	Drying the sample at 60 °C for 48 h and grinding the dried material, Extracting dried sample (5 g) overnight with 90% ethanol (30 mL) at room temperature (3 times), Filtering ethanol extract and vacuum concentrating at 40 °C, Redissolving the residue in ethanol (10 mL), then diluting with water (10 mL) and acidifying with 3% hydrochloric acid (10 mL), Washing with hexane (3 × 30 mL), Cooling the aqueous portion down to 10°C, adjusting pH 8.5 with ammonium, Extracting with chloroform (3 × 30 mL), Washing with water and evaporating chloroform to get the dried residue, Redissolving in 1 mL chloroform, Separating with a silica Sep-Pak cartridge (Waters) pre-saturated with chloroform, Washing with 5 mL each of chloroform and then with chloroform: methanol (9:1, *v*/*v*) before drying over anhydrous sodium sulphate, Evaporating to dryness.	[42]
Hairy root	Serpentine, vincristine, vindoline, catharanthine, vinblastine, tabersonine, tryptamine and ajmalicine	Freeze-drying and grinding the sample, Ultrasound-assisted extracting at room temperature (RT) in 1 mL of MeOH for 60 min, Centrifuging and filtering to get the extract solution.	[26]

Table 5. Preparation and recovery of bioactive compounds from *C. roseus*.

Plant Part	Compounds	Procedures	References
Whole plant	14′,15′-didehydrocyclovinblastine, 17-deacetoxycyclovinblastine, 17-deacetoxyvinamidine, vinamidin, leurosine, catharine, cycloleurosine, leurosidine and cathachunine	Drying and grinding the plant material, Extracting dried sample with 95% EtOH, subsequently evaporating to get a residue, Redissolving with water and then extraction with $CHCl_3$, Separating $CHCl_3$ fraction using silica gel column chromatography (200–300 mesh, Qingdao Marine Chemical Factory, Qingdao, China), then using ODS column (YMC, Kyoto, Japan) to get subfractions, Purifying subfractions by preparative HPLC on a COSMOSIL C18 preparative column (5 µm, 20 × 250 mm, Nacalai Tesque, Kyoto, Japan).	[30,31]
Whole plant	Catharoseumine	Air-drying and grinding the plant material, Extracting dried sample with MeOH under reflux, subsequently evaporating to get a residue, Redissolving with water and adjusting to pH 3 with tartaric acid, Defatting by petroleum ether, and then subjecting to cation ion exchange resins, Washing the resins by water and basified, then eluting with EtOAc and MeOH, The EtOAc fraction was subjected to silica gel column chromatography eluting with $CHCl_3/CH_3OH$ (100:1→1:1) to give 5 fractions (A–E). Fraction A was further applied to column chromatography over reverse-phase C-18 silica gel, Sephadex LH-20, and silica gel chromatography to get catharoseumine.	[28]
Leaf	Vindogentianine	Drying the leaf at 40 °C and grinding dried sample, Defatting dried sample (1 kg) using *n*-hexane (10 L) for 3 days at RT, then removing *n*-hexane to get the residue, The residue was first wetted with 25% ammonia for 1 h, followed by soaking twice with dichloromethane (10 L) for 3 days at RT, Filtering and drying dichloromethane extract under reduced pressure, Acid-base extracting dichloromethane extract using 5% hydrochloric acid and 25% ammonia solution to get an alkaloid crude extract, Separating alkaloid crude extract using silica column chromatography (CC, diameter: 10cm; Merck, Kenilworth, NJ, USA), then flushing using a solvent mixture of CH_2Cl_2 and MeOH (a ratio of 40:60, 20:80 (*v*/*v*)) before 100% MeOH to get fractions (1–15). Applying fraction 4 to preparative thin layer chromatography silica gel F_{254} (1 mm; Merck) under ammonia vapour to get vindogentianine.	[43]
Leaf, stem and root	Ajmaline, yohimbine, vindesine, ajmalicine, serpentine, vincristine, vinblastine, vindoline, vinpocetine and reserpine	Drying the samples under shade and grinding dried sample, Ultrasonic extraction with ethanol for 30 min at 30 °C (sample-to-solvent ratio of 1/10, g/mL) (53 KHz, Bandelin SONOREX, Berlin) and then kept at RT for 24 h, The extracts were filtered through filter paper (Whatman No. 1) and filtrates were collected. The residues were re-extracted with fresh solvent again. The combined filtrates were evaporated to dryness under reduced pressure.	[24]
Leaf, stem, seed and petal	Caffeoylquinic acids, quercetin, kaempferol, isorhamnetin and their derivatives	Freeze-drying sample, Ultrasound-assisted extracting using methanol:water (1:1) as the extraction solvent for 1 h, macerating for 15 h and ultrasonicating again for 1 h (leaf or stem/solvent: 1/10 g/mL, petal/solvent: 1/100 g/mL, seed/solvent: 1/3 g/mL), Centrifuging, filtering and then lyophilising to obtain the powdered phenolic extract.	[32]
Leaf, stem, seed and petal	Caffeoylquinic acids, quercetin, kaempferol, isorhamnetin and their derivatives	Freeze-drying sample, Extraction with boiling water for 20 min (sample-to-solvent ratio of 1/200 g/mL), Filtering and then lyophilising to obtain the powdered phenolic extract.	[33]

MeOH: Methanol. EtOH: Ethanol. EtOAc: Ethyl acetate. RT: Room temperature.

4.2. Recovery of Bioactive Compounds from C. roseus

There have been numerous extraction techniques used for the recovery of bioactive compounds from *C. roseus*, including conventional extraction, ultrasonic-assisted extraction and Soxhlet extraction (Table 5) using the common solvents methanol and ethanol. Soaking materials with 90% ethanol and leaving the mixture overnight has been used to extract vindoline, catharanthine, vincristine and vinblastine from *C. roseus* leaf (Table 5) [42]. To obtain the phenolic extract, lyophilized and powdered plant material (leaves, stems, seeds and petals) were extracted with boiling water for 20 min [33]. In this method, the solvent diffuses into the material matrix, dissolves solutes and liberates them from the plant matrix. This is driven by a concentration gradient at the solid-liquid interphase that exists until the equilibrium is established [44]. This method is cheap and simple but requires prolonged extraction times that may lead to the loss of active compounds through oxidation, ionization and hydrolysis. The Soxhlet extraction was used to prepare the *C. roseus* hairy root extract for isolation of vindoline, ajmalicine, serpentine and catharanthine [41]. Although this method is simple, well established and inexpensive, it is also time consuming and requires large quantities of solvent. Further, it occurs at the boiling point of solvents over a long period that may cause the thermal degradation of bioactive compounds [45].

Ultrasonic extraction is an emerging extraction technique with numerous advantages over conventional thermal extraction methods including shorter extraction times, higher extraction yields, energy and solvent cost savings. *C. roseus* has been subjected to ultrasonic extraction to obtain specific target compounds (Table 5) [24,26,32,40,46]. Other advanced extraction techniques such as microwave-assisted extraction (MAE), enzyme-assisted extraction, pulsed electric field extraction, pressurised liquid extraction and supercritical fluid extraction, which can also reduce extraction time, solvent consumption and environmental pollution could be considered; however, industrial up-scaling potential needs to be taken into consideration.

To obtain phenolic extracts from *C. roseus*, Ferreres et al. [32] and Pereira et al. [33] applied different extraction methods that are ultrasound-assisted extraction and maceration using methanol:water (1/1) as the extraction solvent at room temperature for 17 h versus extraction with boiling water for 20 min (Table 5). In the latter method, water—a cheap, accessible and environmentally friendly solvent—was used, and shorter extraction time was applied as the extraction process was conducted at the boiling point, while the 50% aqueous methanol was used in the former one. However, the phenolic extraction efficiency of these methods could not be fully clarified as the quantity of individual phenolic compounds has not been determined in the former study. *C. roseus* crude extracts could be further separated using chloroform or using ion exchange resins to collect fractions that would be subjected to silica gel column chromatography to collect subfractions, then the individual compounds further purified using column chromatography over Sephadex LH-20 and preparative TLC or preparative HPLC [28,30,31,42]. The detailed recovery process of bioactive compounds from *C. roseus* is presented in Table 5. Of note, besides extraction techniques various extraction parameters, such as temperature, time, solvents, solvent-to-sample ratio, particle size, solvent pH and pressure have been found to significantly affect extraction efficiency and stability of bioactive compounds from plant materials [40,46–50], future studies are recommended to investigate the optimal extraction conditions for recovery of groups or individual alkaloids and/or phenolic compounds from *C. roseus* for further extraction and isolation. In addition, advanced techniques should be applied to identify and test the potential use of unknown compounds in *C. roseus*.

5. Potential Use for Health Benefits

5.1. Potential Use as an Anticancer Agent

C. roseus has been found to contain a range of alkaloids possessing anticancer activity including vinblastine, vincristine, vindoline, vindolidine, vindolicine, vindolinine and vindogentianine [27,43,51]. They inhibit cell proliferation through changing the microtubular dynamics, which induces

apoptosis [52]. Of these, vinblastine and vincristine were the first plant-derived anticancer agents deployed for clinical use [51]. Vinblastine sulphate has been utilised for treatment of Hodgkin's disease, lymphosarcoma, choriocarcinoma, neuroblastoma, carcinoma of breast and lungs and other organs in acute and chronic leukaemia. Vincristine sulphate, an oxidised form of vinblastine [52] arrests mitosis in the metaphase and is effective in treating acute leukaemia (in children), lymphocytic leukaemia, Hodgkin's disease, Wilkins's tumour, neuroblastoma and reticulum cell sarcoma [2].

A range of other indole alkaloids extracted from *C. roseus* has been found to exhibit potent cytotoxic activity against various cancer cell lines. Catharoseumine, a new monoterpenoid indole alkaloid isolated from the *C. roseus* whole plant was found to possess an inhibitory effect on the human promyelocytic leukaemia HL-60 cell line with an IC_{50} of 6.28 μM [28]. Moreover, three newly isolated dimeric indole alkaloids including 14′,15′-didehydrocyclovinblastine, 17-deacetoxycyclovinblastine, 17-deacetoxyvinamidine and five known compounds (vinamidine, leurosine, catharine, cycloleurosine and leurosidine) possessed in vitro inhibition of cell viability against the human breast cancer cell line MDA-MB-231 with an IC_{50} range of 0.73–10.67 μM [30]. Importantly, cathachunine, a new bisindole alkaloid from *C. roseus*, displayed antitumour effects on human leukaemia cells, but at much lower cytotoxicity against normal human endothelial cells, indicating that its activity inhibited selectively toward leukaemia cells.

Along with the studies on anticancer activity of individual alkaloids from *C. roseus*, effect of the entire crude extract on various cancer cell lines was also investigated. Recent findings found that *C. roseus* root and stem extract possessed strong in vitro cytotoxic activity against a panel of cancer cell lines [53,54]. Similarly, the study of Fernández-Pérez et al. [55] confirmed that the powerful antitumor activity of the indole alkaloid-enriched extract obtained from *C. roseus* cell cultures did not result from the effect of a single compound, but depended on the joint action of bioactive compounds. These results revealed the synergistic effect and positive interaction of the bioactive components present in *C. roseus* against cancer cells, which has also been found in other plant materials [56,57] and considered as a strategy for cancer treatment [58]. This phenomenon is possible due to the assistance of compounds with little or no activity to the main active compounds through helping the active component reach the target by improving bioavailability, decreasing metabolism and excretion, complementing mechanisms of action, reversal of resistance and modulating side effects [59].

5.2. Potential Use as an Antidiabetic Agent

C. roseus has been traditionally used as a treatment for diabetes in many regions of the world (Table 1). Juice from the leaf of *C. roseus* was reported to produce a dose-dependent reduction in blood glucose of both normal and diabetic rabbits [60]. The whole plant *C. roseus* methanolic extract displayed effective antihyperglycaemic activity, correlating with improvement in body weight, lipid profile and regeneration of β-cells of the pancreas in diabetic rats [61]. Recently, Tiong et al. [27] investigated the in vitro antidiabetic properties of four pure alkaloids including vindoline, vindolidine, vindolicine and vindolinine isolated from *C. roseus* leaf via the assays of 2-NBDG glucose uptake and inhibition of PTP-1B, an enzyme that regulates negatively the insulin signalling pathway. Improving glucose uptake in pancreatic or muscle cells could amend the hyperglycaemic conditions of type 2 diabetes. It was found that the four alkaloids increased the glucose uptake in mouse β-TC6 pancreatic and mouse myoblast C2C12 cells, coupled with their inhibition of PTP-1B. Among them, vindolicine showed the highest activity. The results supported the traditional utilisation of *C. roseus* for diabetic treatment, highlighting that *C. roseus* is a potent source for further exploring antidiabetic agents.

5.3. Potential Use as Anti-Alzheimer's Disease Agents

Alzheimer's disease (AD) is a neurodegenerative disorder of the central nervous system and accounts for 50–60% of dementia in patients [62]. It is characterised by profound memory impairment, emotional disturbance and personality changes in the late stages of life [63]. Cholinergic neurons in the neocortex and hippocampus are suggested to be predominantly affected in AD, resulting in the cholinergic

hypothesis, which associates AD symptoms to cholinergic deficiency. Therefore, symptomatic treatment for AD has been focused upon augmenting brain cholinergic neurotransmission [64]. The current effective AD therapy is to increase acetylcholine (Ach) levels by inhibiting acetylcholinesterase (AchE), an enzyme responsible for degrading Ach in the synaptic cleft [62,64]. The aqueous extract of *C. roseus* leaf, stem and root has been shown to effectively inhibit AchE in an in vitro microassay [33,65]. Additionally, serpentine, an alkaloid present in *C. roseus* leaf, stem and root [24] displayed a strong activity against AchE with a low IC_{50} value (0.775 μM) [66]. These findings revealed that this plant is a potential source of active compounds for the pharmacological management of neurodegenerative conditions including Alzheimer's disease.

5.4. Other Beneficial Effects

Many microorganisms cause disease in plants, animals and humans, leading to the loss of crops, spoilage of food and increasing problems for human health [67]. Further, microbial resistance, which has become a rising challenging for agriculture and human health, requires more effective antimicrobial agents [52]. Ethanol extracts of *C. roseus* leaf, stem, flower and root have been reported to display antibacterial activity against various bacteria [68,69]. Additionally, the saponin-enriched fractions from *C. roseus* stem and root revealed their potent antifungal activity against *Candida albicans* and *Aspergillus niger* [53,54]. Moreover, individual compounds isolated from *C. roseus* were identified as potent antimicrobial agents. Yohimbine, an alkaloid isolated from *C. roseus* leaf, stem and root [24] was found to exhibit not only antibacterial, antifungal activities but also antiviral activity against herpes simplex virus (type 1) [70]. Remarkably, catharoseumine present in *C. roseus* whole plant was found to inhibit *Plasmodium falciparum* falcipain-2, a protozoan parasite that causes malaria, with an IC_{50} value of 4.06 μM [28].

Wound healing is a complex and dynamic process of restoring cellular structures and tissue layers in damaged tissue as closely as possible to its normal state. The ethanol extracts of *C. roseus* flower and leaf has been demonstrated to possess wound healing activity in rats [71,72], resulting from the astringent and antimicrobial properties of bioactive components present in the *C. roseus* extract [73]. In particular, the leaf extract promoted wound healing in diabetic rats [71]. The results coincided with the findings of Singh et al. [73] who found that the methanol extract of *C. roseus* leaf significantly increased wound contraction in streptozotocin induced diabetic mice. The wound healing activity of *C. roseus* extracts is promising to overcome the poor wound healing typical in the diabetic condition. In these instances, wounds commonly slow healing and can exist for weeks, raising significant challenges for wound care and control in clinical practice.

The leaf extract of *C. roseus* was shown to possess hypotensive and lipid lowering effects in adrenaline-induced hypertensive rats [74]. Ajmalicine found naturally in *C. roseus* [24] has been considered as a hypotensive agent as it acted as an α-adrenergic receptor antagonist [52,75]. Additionally, *C. roseus* extracts and isolated alkaloids including vindoline, vindolidine, vindolicine and vindolinine were found to possess antioxidant properties [27,46,53,54], which could reduce and prevent the oxidation of other molecules. Although oxidation reactions are crucial for life, they produce free radicals and then start a cascade of biochemical reactions, causing oxidative stress that damage or kill cells and contribute to cell ageing, cardiovascular disease, brain disease (associated with Alzheimer's disease), mutagenic changes and cancerous tumour growth [76,77].

6. Conclusions

C. roseus can be considered as a rich source of alkaloids and phenolics, which possess diverse biological properties including anticancer, antidiabetic, antioxidant, antimicrobial and antihypertensive activities. Numerous alkaloids and phenolics have been identified in this material but many compounds are unknown. Consequently, the identification and isolation of new phytochemicals within the different structural components of *C. roseus* should be continued. In addition, potential uses of bioactive compounds derived from this material need to be further investigated for applications in the nutraceutical and pharmaceutical industries.

Author Contributions: Conceptualization, H.N.T.P., Q.V.V., M.C.B. and C.J.S.; Supervision, Q.V.V., M.C.B. and C.J.S.; Writing—original draft, H.N.T.P.; and Writing—review and editing, Q.V.V., M.C.B. and C.J.S. All authors have read and agreed to the published version of the manuscript.

Funding: This work received no external funding.

Acknowledgments: The authors thank the University of Newcastle, Australia for granting Hong Ngoc Thuy Pham the scholarships (UNIPRS and UNRSC 50:50) in 2015.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Easmin, M.S.; Sarker, M.Z.I.; Ferdosh, S.; Shamsudin, S.H.; Yunus, K.B.; Uddin, M.S.; Sarker, M.M.R.; Akanda, M.J.H.; Hossain, M.S.; Khalil, H.P.S.A. Bioactive compounds and advanced processing technology: *Phaleria macrocarpa* (sheff.) Boerl, a review. *J. Chem. Technol. Biotechnol.* **2015**, *90*, 981–991. [CrossRef]
2. Aslam, J.; Khan, S.H.; Siddiqui, Z.H.; Fatima, Z.; Maqsood, M.; Bhat, M.A.; Nasim, S.A.; Ilah, A.; Ahmad, I.Z.; Khan, S.A. *Catharanthus roseus* (L.) G. Don. an important drug: It's applications and production. *Pharm. Glob. (IJCP)* **2010**, *4*, 1–16.
3. Cragg, G.M.; Newman, D.J. Plants as a source of anti-cancer agents. *J. Ethnophamacol.* **2005**, *1*, 72–79. [CrossRef] [PubMed]
4. Pham, H.N.T.; Nguyen, V.T.; Vuong, Q.V.; Bowyer, M.C.; Scarlett, C.J. Effect of extraction solvents and drying methods on the physicochemical and antioxidant properties of *Helicteres hirsuta* Lour. leaves. *Technologies* **2015**, *3*, 285–301. [CrossRef]
5. Pham, H.N.T.; Nguyen, V.T.; Vuong, Q.V.; Bowyer, M.C.; Scarlett, C.J. Bioactive compound yield and antioxidant capacity of *Helicteres hirsuta* Lour. stem as affected by various solvents and drying methods. *J. Food Process. Preserv.* **2017**, *41*, e12879. [CrossRef]
6. Nguyen, V.T.; Pham, N.M.Q.; Vuong, Q.V.; Bowyer, M.C.; van Altenaa, I.A.; Scarlett, C.J. Phytochemical retention and antioxidant capacity of xao tam phan (*Paramignya trimera*) root as prepared by different drying methods. *Dry. Technol.* **2015**, *34*, 324–334. [CrossRef]
7. Vuong, Q.V.; Zammit, N.; Munro, B.R.; Murchie, S.; Bowyer, M.C.; Scarlett, C.J. Effect of drying conditions on physicochemical and antioxidant properties of *Vitex agnus-castus* leaves. *J. Food Process. Preserv.* **2015**, *39*, 2562–2571. [CrossRef]
8. Goli, A.H.; Barzegar, M.; Sahari, M.A. Antioxidant activity and total phenolic compounds of pistachio (*Pistachia vera*) hull extracts. *Food Chem.* **2005**, *92*, 521–525. [CrossRef]
9. Nguyen, V.T.; Bowyer, M.C.; Vuong, Q.V.; van Altena, I.A.; Scarlett, C.J. Phytochemicals and antioxidant capacity of Xao tam phan (*Paramignya trimera*) root as affected by various solvents and extraction methods. *Ind. Crops Prod.* **2015**, *67*, 192–200. [CrossRef]
10. Mehmood, A.; Ishaq, M.; Zhao, L.; Yaqoob, S.; Safdar, B.; Nadeem, M.; Munir, M.; Wang, C. Impact of ultrasound and conventional extraction techniques on bioactive compounds and biological activities of blue butterfly pea flower (*Clitoria ternatea* L.). *Ultrason. Sonochem.* **2019**, *51*, 12–19. [CrossRef]
11. Uribe, E.; Delgadillo, A.; Giovagnoli-Vicuña, C.; Quispe-Fuentes, I.; Zura-Bravo, L. Extraction techniques for bioactive compounds and antioxidant capacity determination of Chilean papaya (*Vasconcellea pubescens*) fruit. *J. Chem.* **2015**, *2015*, 1–8. [CrossRef]
12. Singh, B.; Sangwan, P. Taxonomy, ethnobotany and antimicrobial activity of *Alstonia scholaris* (L.) R. Br., *Carissa carandas* L. and *Catharanthus roseus* (L.) G. Don. *Int. J. Biotech Biosci.* **2011**, *1*, 102–112.

13. Holdsworth, D.K. Traditional medicinal plants of rarotonga, cook islands part I. *Int. J. Crude Drug Res.* **1990**, *28*, 209–218.
14. Khan, M.H.; Yadava, P. Antidiabetic plants used in Thoubal district of Manipur, Northeast India. *Indian J. Tradit. Know.* **2010**, *9*, 510–514.
15. Marles, R.J.; Farnsworth, N.R. Antidiabetic plants and their active constituents. *Phytomedicine* **1995**, *2*, 137–189.
16. Swanston-Flatt, S.K.; Day, C.; Flatt, P.R.; Gould, B.J.; Bailey, C. Glycaemic effects of traditional European plant treatments for diabetes. Studies in normal and streptozotocin diabetic mice. *Diabetes Res.* **1989**, *10*, 69–73.
17. Vo, V.C. Dictionary of Vietnamese medicinal plants, Medical Publishing House, Ha Noi. *Am. J. Plant Sci.* **2012**, *4*, 210–215.
18. Ochwang'i, D.O.; Kimwele, C.N.; Oduma, J.A.; Gathumbi, P.K.; Mbaria, J.M.; Kiama, S.G. Medicinal plants used in treatment and management of cancer in Kakamega County, Kenya. *J. Ethnopharmacol.* **2014**, *151*, 1040–1055.
19. Muthu, C.; Ayyanar, M.; Raja, N.; Ignacimuthu, S. Medicinal plants used by traditional healers in Kancheepuram district of Tamil Nadu, India. *J. Ethnobiol. Ethnomed.* **2006**, *2*, 43.
20. Fernandes, L.; Van Rensburg, C.; Hoosen, A.; Steenkamp, V. In vitro activity of medicinal plants of the Venda region, South Africa, against *Trichomonas vaginalis*. *S. Afr. J. Epidemiol. Infect.* **2008**, *23*, 26–28.
21. Semenya, S.; Potgieter, M. *Catharanthus roseus* (L.) G. Don.: Extraordinary bapedi medicinal herb for gonorrhoea. *J. Med. Plant. Res.* **2013**, *7*, 1434–1438.
22. Chigora, P.; Masocha, R.; Mutenheri, F. The role of indigenous medicinal knowledge (IMK) in the treatment of ailments in rural Zimbabwe: The case of Mutirikwi communal lands. *J. Sustain. Dev. Afr.* **2007**, *9*, 26–43.
23. Wansi, J.D.; Devkota, K.P.; Tshikalange, E.; Kuete, V. Alkaloids from the medicinal plants of Africa. In *Medicinal Plant Research in Africa*; Kuete, V., Ed.; Elsevier: Oxford, UK, 2013; pp. 557–605.
24. Kumar, S.; Singh, A.; Kumar, B.; Singh, B.; Bahadur, L.; Lal, M. Simultaneous quantitative determination of bioactive terpene indole alkaloids in ethanolic extracts of *Catharanthus roseus* (L.) G. Don by ultra high performance liquid chromatography–tandem mass spectrometry. *J. Pharm. Biomed. Anal.* **2018**, *151*, 32–41. [CrossRef] [PubMed]
25. Mu, F.S.; Yang, L.Q.; Wang, W.; Luo, M.; Fu, Y.J.; Guo, X.R.; Zu, Y.G. Negative-pressure cavitation extraction of four main vinca alkaloids from *Catharanthus roseus* leaves. *Molecules* **2012**, *17*, 8742–8752. [CrossRef] [PubMed]
26. Tikhomiroff, C.; Jolicoeur, M. Screening of *Catharanthus roseus* secondary metabolites by high-performance liquid chromatography. *J. Chromatogr. A* **2002**, *995*, 87–93. [CrossRef]
27. Tiong, S.H.; Looi, C.Y.; Hazni, H.; Arya, A.; Paydar, M.; Wong, W.F.; Cheah, S.C.; Mustafa, M.R.; Awang, K. Antidiabetic and antioxidant properties of alkaloids from *Catharanthus roseus* (L.) G. Don. *Molecules* **2013**, *18*, 9770–9784. [CrossRef]
28. Wang, L.; He, H.-P.; Di, Y.-T.; Zhang, Y.; Hao, X.-J. Catharoseumine, a new monoterpenoid indole alkaloid possessing a peroxy bridge from *Catharanthus roseus*. *Tetrahedron Lett.* **2012**, *53*, 1576–1578.
29. El-Sayed, A.; Cordell, G.A. Catharanthus alkaloids. XXXIV. Catharanthamine, a new antitumor bisindole alkaloid from *Catharanthus roseus*. *J. Nat. Prod.* **1981**, *44*, 289–293. [CrossRef]
30. Wang, C.-H.; Wang, G.-C.; Wang, Y.; Zhang, X.-Q.; Huang, X.-J.; Zhang, D.-M.; Chen, M.-F.; Ye, W.-C. Cytotoxic dimeric indole alkaloids from *Catharanthus roseus*. *Fitoterapia* **2012**, *83*, 765–769. [CrossRef]
31. Wang, X.-D.; Li, C.-Y.; Jiang, M.-M.; Li, D.; Wen, P.; Song, X.; Chen, J.-D.; Guo, L.-X.; Hu, X.-P.; Li, G.-Q. Induction of apoptosis in human leukemia cells through an intrinsic pathway by cathachunine, a unique alkaloid isolated from *Catharanthus roseus*. *Phytomedicine* **2016**, *23*, 641–653. [CrossRef]
32. Ferreres, F.; Pereira, D.M.; Valentao, P.; Andrade, P.B.; Seabra, R.M.; Sottomayor, M. New phenolic compounds and antioxidant potential of *Catharanthus roseus*. *J. Agr. Food Chem.* **2008**, *56*, 9967–9974. [CrossRef] [PubMed]
33. Pereira, D.M.; Ferreres, F.; Oliveira, J.; Valentao, P.; Andrade, P.B.; Sottomayor, M. Targeted metabolite analysis of *Catharanthus roseus* and its biological potential. *Food Chem. Toxicol.* **2009**, *47*, 1349–1354. [CrossRef]
34. Misra, N.; Gupta, A.K. Effect of salinity and different nitrogen sources on the activity of antioxidant enzymes and indole alkaloid content in *Catharanthus roseus* seedlings. *J. Plant Physiol.* **2006**, *163*, 11–18. [CrossRef] [PubMed]
35. Zheng, Z.; Wu, M. Cadmium treatment enhances the production of alkaloid secondary metabolites in *Catharanthus roseus*. *Plant Sci.* **2004**, *166*, 507–514. [CrossRef]

36. Binder, B.Y.; Peebles, C.A.; Shanks, J.V.; San, K.Y. The effects of UV-B stress on the production of terpenoid indole alkaloids in *Catharanthus roseus* hairy roots. *Biotechnol. Prog.* **2009**, *25*, 861–865. [CrossRef] [PubMed]
37. Mishra, M.R.M.; Srivastava, R.K.; Akhtar, N. Effect of nitrogen, phosphorus and medium ph to enhance alkaloid production from *Catharanthus roseus* cell suspension culture. *Int. J. Second. Metab.* **2019**, *6*, 137–153. [CrossRef]
38. Oueslati, S.; Karray-Bouraoui, N.; Attia, H.; Rabhi, M.; Ksouri, R.; Lachaal, M. Physiological and antioxidant responses of *Mentha pulegium* (Pennyroyal) to salt stress. *Acta Physiol. Plant.* **2010**, *32*, 289–296. [CrossRef]
39. Mahdavi, A.; Moradi, P.; Mastinu, A. Variation in terpene profiles of *Thymus vulgaris* in water deficit stress response. *Molecules* **2020**, *25*, 1091. [CrossRef]
40. Pham, H.N.T.; Vuong, Q.V.; Bowyer, M.C.; Scarlett, C.J. Effect of extraction solvents and thermal drying methods on bioactive compounds and antioxidant properties of *Catharanthus roseus* (L.) G. Don (Patricia White cultivar). *J. Food Process. Preserv.* **2017**, *41*, e13199. [CrossRef]
41. Bhadra, R.; Vani, S.; Shanks, J.V. Production of indole alkaloids by selected hairy root lines of *Catharanthus roseus*. *Biotechnol. Bioeng.* **1993**, *41*, 581–592. [CrossRef]
42. Uniyal, G.; Bala, S.; Mathur, A.; Kulkarni, R. Symmetry C18 column: A better choice for the analysis of indole alkaloids of *Catharanthus roseus*. *Phytochem. Anal.* **2001**, *12*, 206–210. [CrossRef] [PubMed]
43. Tiong, S.H.; Looi, C.Y.; Arya, A.; Wong, W.F.; Hazni, H.; Mustafa, M.R.; Awang, K. Vindogentianine, a hypoglycemic alkaloid from *Catharanthus roseus* (L.) G. Don (Apocynaceae). *Fitoterapia* **2015**, *102*, 182–188. [CrossRef] [PubMed]
44. Kha, T.C.; Nguyen, M.H. Extraction and isolation of plant bioactives. In *Plant Bioactive Compounds for Pancreatic Cancer Prevention and Treatment*; Scarlett, C.J., Vuong, Q.V., Eds.; Nova Science Publishers: New York, NY, USA, 2014; pp. 117–144.
45. Wang, L.; Weller, C.L. Recent advances in extraction of nutra-ceuticals from plants. *Trends Food Sci. Technol.* **2006**, *17*, 300–312. [CrossRef]
46. Pham, H.N.T.; Vuong, Q.V.; Bowyer, M.C.; Scarlett, C.J. Ultrasound-assisted extraction of *Catharanthus roseus* (L.) G. Don (Patricia White cultivar) stem for maximizing saponin yield and antioxidant capacity. *J. Food Process. Preserv.* **2018**, *42*, e13597. [CrossRef]
47. Lucas-González, R.; Fernández-López, J.; Pérez-Álvarez, J.Á.; Viuda-Martos, M. Effect of particle size on phytochemical composition and antioxidant properties of two persimmon flours from *Diospyros kaki* Thunb. vars.'Rojo Brillante' and 'Triumph'co-products. *J. Sci. Food Agri.* **2018**, *98*, 504–510. [CrossRef]
48. Zaiter, A.; Becker, L.; Karam, M.-C.; Dicko, A. Effect of particle size on antioxidant activity and catechin content of green tea powders. *J. Food Sci. Technol.* **2016**, *53*, 2025–2032. [CrossRef]
49. Boi, V.N.; Cuong, D.X.; Vinh, P.T.K. Effects of extraction conditions over the phlorotannin content and antioxidant activity of extract from brown algae *Sargassum serratum* (Nguyen Huu Dai 2004). *Free Radic. Antioxid.* **2017**, *7*, 115–122. [CrossRef]
50. Alexandre, E.M.; Araújo, P.; Duarte, M.F.; de Freitas, V.; Pintado, M.; Saraiva, J.A. Experimental design, modeling, and optimization of high-pressure-assisted extraction of bioactive compounds from pomegranate peel. *Food Bioprocess Tech.* **2017**, *10*, 886–900. [CrossRef]
51. Cragg, G.M.; Newman, D.J. Plants as a source of anti-cancer and anti-HIV agents. *Ann. Appl. Biol.* **2003**, *143*, 127–133. [CrossRef]
52. Almagro, L.; Fernández-Pérez, F.; Pedreño, M. Indole alkaloids from *Catharanthus roseus*: Bioproduction and their effect on human health. *Molecules* **2015**, *20*, 2973–3000. [CrossRef]
53. Pham, H.N.T.; Sakoff, J.A.; Vuong, Q.V.; Bowyer, M.C.; Scarlett, C.J. Screening phytochemical content, antioxidant, antimicrobial and cytotoxic activities of *Catharanthus roseus* (L.) G. Don stem extract and its fractions. *Biocatal. Agric. Biotechnol.* **2018**, *16*, 405–411. [CrossRef]
54. Pham, H.N.T.; Sakoff, J.A.; Vuong, Q.V.; Bowyer, M.C.; Scarlett, C.J. Phytochemical, antioxidant, anti-proliferative and antimicrobial properties of *Catharanthus roseus* root extract, saponin-enriched and aqueous fractions. *Mol. Biol. Rep.* **2019**, *46*, 3265–3273. [CrossRef] [PubMed]
55. Fernández-Pérez, F.; Almagro, L.; Pedreño, M.A.; Gomez Ros, L.V. Synergistic and cytotoxic action of indole alkaloids produced from elicited cell cultures of *Catharanthus roseus*. *Pharm. Biol.* **2013**, *51*, 304–310. [CrossRef] [PubMed]

56. Bhuyan, D.J.; Vuong, Q.V.; Bond, D.R.; Chalmers, A.C.; Bowyer, M.C.; Scarlett, C.J. *Eucalyptus microcorys* leaf extract derived HPLC-fraction reduces the viability of MIA PaCa-2 cells by inducing apoptosis and arresting cell cycle. *Biomed. Pharmacother.* **2018**, *105*, 449–460. [CrossRef] [PubMed]
57. Barth, S.W.; Faehndrich, C.; Bub, A.; Watzl, B.; Will, F.; Dietrich, H.; Rechkemmer, G.; Briviba, K. Cloudy apple juice is more effective than apple polyphenols and an apple juice derived cloud fraction in a rat model of colon carcinogenesis. *J. Agric. Food Chem.* **2007**, *55*, 1181–1187. [CrossRef] [PubMed]
58. Jiang, H.; Shang, X.; Wu, H.; Huang, G.; Wang, Y.; Al-Holou, S.; Gautam, S.C.; Chopp, M. Combination treatment with resveratrol and sulforaphane induces apoptosis in human U251 glioma cells. *Neurochem. Res.* **2010**, *35*, 152. [CrossRef]
59. Rasoanaivo, P.; Wright, C.W.; Willcox, M.L.; Gilbert, B. Whole plant extracts versus single compounds for the treatment of malaria: Synergy and positive interactions. *Malar. J.* **2011**, *10*, S4. [CrossRef]
60. Nammi, S.; Boini, M.K.; Lodagala, S.D.; Behara, R.B.S. The juice of fresh leaves of *Catharanthus roseus* Linn. reduces blood glucose in normal and alloxan diabetic rabbits. *BMC Complement. Altern. Med.* **2003**, *3*, 4. [CrossRef]
61. Ahmed, M.F.; Kazim, S.M.; Ghori, S.S.; Mehjabeen, S.S.; Ahmed, S.R.; Ali, S.M.; Ibrahim, M. Antidiabetic activity of *Vinca rosea* extracts in alloxan-induced diabetic rats. *Int. J. Endocrinol.* **2010**, *2010*, 841090. [CrossRef]
62. Yoo, K.-Y.; Park, S.-Y. Terpenoids as potential anti-Alzheimer's disease therapeutics. *Molecules* **2012**, *17*, 3524–3538. [CrossRef]
63. Bartolucci, C.; Perola, E.; Pilger, C.; Fels, G.; Lamba, D. Three-dimensional structure of a complex of galanthamine (Nivalin®) with acetylcholinesterase from *Torpedo californica*: Implications for the design of new anti-Alzheimer drugs. *Proteins* **2001**, *42*, 182–191. [CrossRef]
64. Greenblatt, H.M.; Guillou, C.; Guénard, D.; Argaman, A.; Botti, S.; Badet, B.; Thal, C.; Silman, I.; Sussman, J.L. The complex of a bivalent derivative of galanthamine with torpedo acetylcholinesterase displays drastic deformation of the active-site gorge: Implications for structure-based drug design. *J. Am. Chem. Soc.* **2004**, *126*, 15405–15411. [CrossRef] [PubMed]
65. Pereira, D.M.; Faria, J.; Gaspar, L.; Ferreres, F.; Valentao, P.; Sottomayor, M.; Andrade, P.B. Exploiting *Catharanthus roseus* roots: Source of antioxidants. *Food Chem.* **2010**, *121*, 56–61. [CrossRef]
66. Pereira, D.M.; Ferreres, F.; Oliveira, J.M.A.; Gaspar, L.; Faria, J.; Valentão, P.; Sottomayor, M.; Andrade, P.B. Pharmacological effects of *Catharanthus roseus* root alkaloids in acetylcholinesterase inhibition and cholinergic neurotransmission. *Phytomedicine* **2010**, *17*, 646–652. [CrossRef] [PubMed]
67. Li, Y.; Zhang, J.-J.; Xu, D.-P.; Zhou, T.; Zhou, Y.; Li, S.; Li, H.-B. Bioactivities and health benefits of wild fruits. *Int. J. Mol. Sci.* **2016**, *17*, 1258. [CrossRef] [PubMed]
68. Goyal, P.; Khanna, A.; Chauhan, A.; Chauhan, G.; Kaushik, P. In vitro evaluation of crude extracts of *Catharanthus roseus* for potential antibacterial activity. *Int. J. Green Pharm.* **2008**, *2*, 176–181. [CrossRef]
69. Ramya, S.; Govindaraji, V.; Kannan, K.N.; Jayakumararaj, R. In vitro evaluation of antibacterial activity using crude extracts of *Catharanthus roseus* L.(G.) Don. *Ethnobot. Leafl.* **2008**, *12*, 1067–1072.
70. Özçelik, B.; Kartal, M.; Orhan, I. Cytotoxicity, antiviral and antimicrobial activities of alkaloids, flavonoids, and phenolic acids. *Pharm. Biol.* **2011**, *49*, 396–402. [CrossRef]
71. Nayak, B.; Anderson, M.; Pereira, L.P. Evaluation of wound-healing potential of *Catharanthus roseus* leaf extract in rats. *Fitoterapia* **2007**, *78*, 540–544. [CrossRef]
72. Nayak, B.; Pereira, L.M.P. *Catharanthus roseus* flower extract has wound-healing activity in sprague dawley rats. *BMC Complement. Altern. Med.* **2006**, *6*, 41. [CrossRef]
73. Singh, A.; Singh, P.K.; Singh, R.K. Antidiabetic and wound healing activity of *Catharanthus roseus* L. in streptozotocin-induced diabetic mice. *Am. J. Phytomed. Clin. Ther.* **2014**, *2*, 686–692.
74. Ara, N.; Rashid, M.; Amran, S. Comparison of hypotensive and hypolipidemic effects of *Catharanthus roseus* leaves extract with atenolol on adrenaline induced hypertensive rats. *Pak. J. Pharm. Sci.* **2009**, *22*, 267–271. [PubMed]
75. Wink, M.; Schmeller, T.; Latz-Brüning, B. Modes of action of allelochemical alkaloids: Interaction with neuroreceptors, DNA, and other molecular targets. *J. Chem. Ecol.* **1998**, *24*, 1881–1937. [CrossRef]
76. Apak, R.; Güçlü, K.; Özyürek, M.; Çelik, S.E. Mechanism of antioxidant capacity assays and the CUPRAC (cupric ion reducing antioxidant capacity) assay. *Microchim. Acta* **2008**, *160*, 413–419. [CrossRef]

77. Hamid, A.; Aiyelaagbe, O.; Usman, L.; Ameen, O.; Lawal, A. Antioxidants: Its medicinal and pharmacological applications. *Afr. J. Pure Appl. Chem.* **2010**, *4*, 142–151.