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.)

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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-deacetoxyvinblastine and 17-deacetoxyvinamidine effectively inhibited human cancer cell lines in vitro. Moreover, vindoline, vindolidine, vindoline 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).](image)

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 mellitus        | 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  | Diabetes, hypertension   | Eighteen leaves are boiled in a kettle of water.  | Oral intake                                   | Cook Island                  | [13]       |
| white flowered     | and cancer               | The cool solution is drunk daily.                 |                                               |                              |            |
| varieties          |                          |                                                  |                                               |                              |            |
| Whole plant        | Throat, stomach,         | The whole plant is boiled with water. Found       | Oral intake, Usually taken together with Seabion solum whole plant | Kenya                        | [18]       |
|                    | oesophageal cancer       |                                                  | Applied topically                             |                              |            |
| 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,  | The whole plant is boiled with water.             | Oral intake                                   | Vietnam                      | [17]       |
|                    | dysentery, cancer        |                                                  |                                               |                              |            |

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, norlupanine 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 | ![Vincristine](image) | [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 | ![Vinblastine](image) | [24]       |
|           | 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 | ![Vinpocetine](image) | [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 | ![Reserpine](image) | [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   | ![Ajmalicine](image1) | [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   | ![Ajmaline](image2) | [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   | ![Yohimbine](image3) | [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   | ![Vindesine](image4) | [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  | ![Serpentine](image5) | [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  | ![Catharanthine](image6) | [25,26]   |
| Vindolidine     | Leaf       | 0.14%                 | ![Vindolidine](image7) | [24,27]   |
| Vindoline       | Leaf       | 5.301–19.463 mg/g DE  | ![Vindoline](image8) | [24,27]   |
|                 | Stem       | 0.144–3.344 mg/g DE   |                    |            |
|                 | Root       | 0.021–9.690 mg/g DE   |                    |            |
| Vindolicine     | Leaf       | 0.07%                 | ![Vindolicine](image9) | [27]       |
| Vindolinine     | Leaf       | 0.02%                 | ![Vindolinine](image10) | [27]       |
| Catharoseumine  | Whole plant| 0.786 mg/kg           | ![Catharoseumine](image11) | [28]       |
Table 2. Cont.

| Alkaloids                             | Plant Part | Quantity     | Chemical Structure | References |
|---------------------------------------|------------|--------------|--------------------|------------|
| Tabersonine                           | Hairy root | NR           | ![image](image)    | [26]       |
| Tryptamine                            | Hairy root | NR           | ![image](image)    | [26]       |
| Catharanthamine                       | Leaf       | NR           | ![image](image)    | [29]       |
| 14′,15′-Didehydrocyclovincristine     | Whole plant| 0.071 mg/g DE| ![image](image)    | [30]       |
| 17-Deacetoxycyclovincristine          | Whole plant| 0.089 mg/g DE| ![image](image)    | [30]       |
| Cycloleurosine                        | Whole plant| 0.116 mg/g DE| ![image](image)    |            |
| 17-Deacetoxyvinamidine                | Whole plant| 0.107 mg/g DE| ![image](image)    | [30]       |
| Vinamidine                            | Whole plant| 0.071 mg/g DE| ![image](image)    |            |
| Leurosine                             | Whole plant| 0.134 mg/g DE| ![image](image)    | [30]       |
| Leurosidine                           | Whole plant| 0.107 mg/g DE| ![image](image)    | [30]       |
| Catharanthine                         | Whole plant| 0.098 mg/g DE| ![image](image)    |            |
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]    |
| Stem        | 4-O-caffeoylquinic acid                         | 2874.6 ± 151.6              | [32,33]    |
| Stem        | 5-O-caffeoylquinic acid                         | 22.5 ± 1.5                  | [32,33]    |
| Stem        | Quercetin-3-O(2,6-di-O-rhamnosyl-galactoside)   | 190.5 ± 3.1                 | [32,33]    |
| Stem        | Kaempferol-3-O(2,6-di-O-rhamnosyl-galactoside)  | 190.8 ± 5.3                 | [32,33]    |
| Stem        | Isoisorhamnatin-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]    |
| Leaf        | Kaempferol-3-O(2,6-di-O-rhamnosyl-galactoside)-7-O-hexoside | 52.7 ± 1.0                  | [32,33]    |
| Leaf        | 4-O-caffeoylquinic acid                         | 5150.6 ± 137.2              | [32,33]    |
| Leaf        | 5-O-caffeoylquinic acid                         | 187.7 ± 0.5                 | [32,33]    |
| Leaf        | Quercetin-3-O(2,6-di-O-rhamnosyl-galactoside)   | 310.9 ± 5.0                 | [32,33]    |
| Leaf        | 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]    |
| Seeds       | Kaempferol-3-O(2,6-di-O-rhamnosyl-galactoside)  | 582.7 ± 6.6                 | [32,33]    |
| Seeds       | Quercetin-3-O(2,6-di-O-rhamnosyl-galactoside)   | 2714.2 ± 4.3                | [32,33]    |
| Seeds       | Kaempferol-3-O(2,6-di-O-rhamnosyl-glucoside)    | 56.6 ± 0.4                  | [32,33]    |
| Seeds       | Isoisorhamnatin-3-O(2,6-di-O-rhamnosyl-glucoside) | 354.1 ± 8.2                 | [32,33]    |
| Seeds       | Kaempferol-3-O(6-O-rhamnosyl-galactoside)       | 112.1 ± 16.0                | [32,33]    |
| Seeds       | Isoisorhamnatin-3-O(6-O-rhamnosyl-glucoside)    | 372.0 ± 65.2                | [32,33]    |
| Petal       | 4-O-caffeoylquinic acid                         | 11153.2 ± 126.4             | [32,33]    |
| Petal       | Quercetin-3-O(2,6-di-O-rhamnosyl-galactoside)   | 1027.9 ± 7.0                | [32]       |
| Petal       | Kaempferol-3-O(2,6-di-O-rhamnosyl-galactoside)  | 8120.8 ± 74.4               | [32]       |
| Petal       | Kaempferol-3-O(2,6-di-O-rhamnosyl-glucoside)    | 4296.3 ± 34.4               | [32]       |
| Petal       | Kaempferol-3-O(6-O-rhamnosyl-galactoside)       | 9567.2 ± 98.5               | [32,33]    |
| Petal       | Kaempferol-3-O(6-O-rhamnosyl-glucoside)         | 4639.8 ± 21.9               | [32,33]    |
| Petal       | Isoisorhamnatin-3-O(6-O-rhamnosyl-galactoside)  | 989.2 ± 33.0                | [32,33]    |
| Petal       | Isoisorhamnatin-3-O(6-O-rhamnosyl-glucoside)    | 1330.4 ± 10.8               | [32,33]    |
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 (NH₄)₂HPO₄ solution, Fractionating using a 300-mg C₁₈ Maxi-Clean cartridge, then eluting successively with 4 mL of mixture of MeOH:5 mM (NH₄)₂HPO₄ (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-deacetoxyvinblastine, 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, subsequent redissolving with water and then extraction with CHCl₃, separating CHCl₃ 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, subsequently evaporating to get a residue, defatting by petroleum ether, and then subjecting to cation 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 CHCl₃/CH₃OH (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 CH₂Cl₂ 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 F₂₅₄ (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, 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 ultrasonating 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, 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, Wilkin’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, vindolinine 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.

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