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A Review: Phytochemical Screening and Antioxidant Activity from Several Parts of *Gardenia jasminoides* J. Ellis

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Abstract
Background*: Gardenia jasminoides J. Ellis belongs to the Rubiaceae plant group originating from China known as zhi zi. In China, the compound crocetin contained in the fruit of this plant has long been used as a natural yellow dye. In Indonesia, this plant is traditionally used to treat fever, sprue, constipation, diabetes mellitus, and to improve blood circulation.*
Methods*: The method of reviewing this article is to explore several search sites such as Science Direct, Researchgate, and Google Scholar by looking for sources or literature in the form of primary data in the form of official books and international journals within the last 20 years (2000-2020).*
Results*: A total of 104 compounds have been isolated and identified from all parts of the gardenia plant. These compounds are phenolics, flavonoids, iridoid glycosides, monoterpenoids, triterpenoids, carotenoids, organic acids, and their derivatives. Geniposide from the group of iridoid glycoside compounds is considered to be a bioactive ingredient that is the main characteristic of gardenia plants. The phenolic compounds contained in gardenia plants have the potential as antioxidants.*
Conclusions*: Gardenia contains compounds that have the potential to be further developed into standardized raw materials and formulated into herbal medicines.*
Keywords*: Gardenia jasminoides J. Ellis, phytochemical screening, antioxidant activity*

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I. Introduction

Gardenia jasminoides J. Ellis is a Rubiaceae plant originating from China known as san tze, zhi zi. In Indonesia, it is known as kaca piring. In Vietnam, it is known as danh danh, chi tur. While in France it is called jasmin du cap. And in India, it is called cape jasmine, gardenia[1]. Because of the fragrance of the flowers, gardenia has a commercial value to be used as perfume. Traditionally, people in Indonesia generally use squeezed water and boiled water from gardenia leaves which are drunk by mixing it with honey, palm sugar, or rock sugar to treat fever, sprue, constipation, and diabetes mellitus[2].

Gardenia is an annual herbaceous plant and has many branches, twigs, and thick leaves. The flowers are large and the tree trunk can reach a height of around 1-2 meters with tap roots[1,3]. Annual shrubs that can reach 2 meters in height. The stem is round, woody, and has many branches. The leaves are oval, thick, smooth, and shiny on the upper surface. A single flower, short-stemmed, white in color, coming out of the tip of the twig, large, similar to white roses with circular crowns and arranged to form a single elegant unit, the smell is fragrant. The shape of the fruit is ovoid, the skin is thin, contains a yellow pigment, and has many seeds[4].

The parts of the gardenia that can be used are fruit, leaves, flowers, and roots. The leaves contain saponins, flavonoids, polyphenols, and essential oils. Fruit contains crocin. In China, the flowers are used as a flavor enhancer in tea leaves. The fruit is edible and can be used as a natural yellow coloring agent in foods such as turmeric[4]. The compound crocetin contained in the fruit has traditionally been used as a spice, food coloring, and herbal medicine[5].

Many of these traditional uses have allowed many researchers to carry out scientific studies focusing on the chemical content, pharmacological activity, mechanism of action of compounds, and their toxicity. One of them reported that geniposide compounds from gardenia fruit parts showed effective results in antiangiogenic assays using the chorioallantoic membrane (CAM) test from the ethanolic extract of gardenia fruit[6]. In this review article, we summarize the results of various studies on phytochemical screening and antioxidant activity tests of several parts of the gardenia plant, namely leaves, fruit, flowers, and roots published in recent years. We hope this article will better understand the potential compounds contained in gardenia plants and can be developed for useful applications.

II. Methods

In compiling this review article, the method used is to use a literature study by looking for sources or literature in the form of primary data in the form of official books and international journals within the last 20 years (2000-2020). In addition, in the preparation of this review article, data were searched using online media with the keywords phytochemical screening and antioxidant activity of *Gardenia jasminoides* J. Ellis. The main reference search used in this review article is through trusted websites such as Science Direct, Researchgate, Google Scholar, and other published and trusted journals.

III. Result and Discussion

Phytochemical Screening

Phytochemistry is the science that describes the chemical aspects of a plant. Phytochemical studies include descriptions covering various organic compounds formed and stored by organisms, namely their chemical structures, biosynthesis, changes and metabolism, natural distribution and biological functions, isolation, and comparison of chemical compositions of various types of plants. The phytochemical analysis is carried out to determine the characteristics of the bioactive components of a crude extract that have toxic effects or other pharmacological effects that are beneficial when tested with biological systems or bioassays[7].

The phytochemical analysis is a part of pharmacognosy that studies methods or methods of analyzing chemical content contained in plants or animals as a whole or their parts, including how to isolate or separate them[8]. Another thing that plays an important role in the phytochemical screening procedure is the solvent for extraction[9]. Difficulties often arise if the choice of solvent is only based on the general provisions of the degree of solubility of a compound under study. This is due to the presence of compounds from other groups in the plant which will affect the solubility process of the desired compound. Each plant certainly has a different composition of content so that the solubility of a compound cannot be determined with certainty. Phytochemical screening of plant simplicia, among others, was to test flavonoid compounds, terpenoids/triterpenoids, alkaloids, steroids, saponins, and tannins[9].

The results of the phytochemical screening of gardenia plants qualitatively showed the similarity of compounds in the leaves and fruit, namely the presence of a class of phenolic compounds, namely flavonoids, rutin hydrate, quercetin, tannins, and glycosides (Table 1). Quantitative analysis of the compound with the highest content is a group of flavonoid compounds. The majority in every part of the gardenia plant obtained high total flavonoids (Table 2).

Table 1. Qualitative phytochemical screening of *Gardenia jasminoides* J. Ellis.

No.	Plant Parts	Compound	Reference
1.	Leaf	Flavonoids	[10]
		Tannins	
		Glycosides	
		Steroids	
		Alkaloids	
		Catechins	
		Rutin	
		Quercetin	
		Gallic acid	
		7β, 8β-epoxy-8α-dihydrogeniposide **(1)**	[11]
		8-epiapodantheroside **(2)**	
		Monotropein metil ester (=galioside) **(3)**	
		Gardenoside (**4)**	
		Deacetylasperulosidic acid methyl ester **(5)**	
		Scandoside methyl ester **(6)**	
		Geniposide**(7)**	
		Ixoroside**(8)**	
		8-O-methylmonotropein methyl ester **(9)**	
		6-O-methyldeacetylaspe-rulosidic acid methyl ester **(10)**	
		6-O-methylscandoside methyl ester **(11)**	
2.	Fruits	Crocin	[12]
		Geniposide	
		Geniposidic acid	[13]
		Secoxyloganin	
		Gardenoside	
		Genipin 1-gentiobioside	
		Scandoside methyl ester	
		Shanzhiside	
		Chlorogenic acid	
		Cryptochlorogenic acid	
		Neochlorogenic acid	

		Isochlorogenic acid	
		Isochlorogenic caffeic acid	
		Crocin-1	
		Crocin-2	
		Genipin	[14]
		Rutin	
		Ursolic acid	
		Crocetin derivate	[15]
		6a-hydroxy geniposide **(1)**	[16]
		6-O-methyl scandoside methyl ester **(2)**	
		6-O-methyl deacetyl asperulosidic acid methyl ester **(3)**	
		Gardenoside **(4)**	
		8-O-methyl monotropein methyl ester **(5)**	
		Shanzhiside **(6)**	
		Gardoside **(7)**	
		10-O-trans-sinapoyl geniposide **(8)**	
		6''-O-trans-p-coumaroyl genipin gentiobioside **(10)**	
		Rehmapicrogenin **(11)**	
		Jasminoside C **(12)**	
		Jasminoside B **(13)**	
		Jasminoside G **(14)**	
		Jasminoside K **(15)**	
		Jasminoside I **(16)**	
		Jasminoside H **(17)**	
		Epijasminoside H **(18)**	
		6'-O-sinapoyl jasminoside C **(19)**	
		6'-O-trans-sinapoyl jasminoside L **(20)**	
3.	Roots	Gardenisida A **(1)**	[17]
		Gardenisida B **(2)**	
		Gardenisida C **(3)**	
		Oleanolic acid 3-O-β-D- glucuronopyranoside-6′-O-methly ester **(4)**	
		Oleanolic acid 3-O-β-D- glucuropyranoside **(5)**	
		Hederagenin 3-O-β-D-glucuronopyranoside-6′-O- methly ester **(6)**	
		Chikusetsusaponin IVa methyl ester **(7)**	
		Chikusetsusaponin **(8)**	
		Chikusetsusaponin IVa butyl ester **(9)**	
		Siaresinolic acid 28-O-β-D-glucopyranosyl ester **(10)**	
4.	Flower	5, 7, 3'-trihydroxy-6, 4', 5'-trimethoxyflavone **(1)**	[18]
		5, 7, 3', 5'- tetrahydroxy-6, 4'-dymethoxyflavone **(2)**	
		Kaempferol **(3)**	
		Quercetin **(4)**	
		3β,23- dihydroxy-urs-12-en-28-oic acid **(5)**	
		3β,19α-dihydroxy-urs-12-en-28-oic acid **(6)**	
		3β,19α,23-trihydroxy-urs-12-en-28-oic acid **(7)**	
		Emodin **(8)**	
		Physcion **(9)**	
		Crocin-I **(10)**	
		β-daucosterol **(11)**	
		β-sitosterol **(12)**	
		Stearic acid **(13)**	
		Palmitic acid **(14)**	
		Oleic acid **(15)**	
		2'-O-trans-coumaroylshanzhiside **(1)**	[19]
		6'-O-trans-coumaroyl-shanzhiside **(2)**	
		8α-butylgardenoside B **(3)**	
		6α-methoxygenipin **(4)**	
		4-methoxy-benzenepropanol-3-O-β-D-glucopyranoside **(5)**	
		Linalool	[20]
		α-farnesene	
		α-terpineol	
		Geraniol	
		Cembrene A	
		Cis-3-hexenyl tiglate	
		Pentacosane	
		Hexyl tiglate	
		Nerol	
		Tricosane	
		Geranyl angelate	
		Tau-cadinol	
		8-hydroxylinalool	

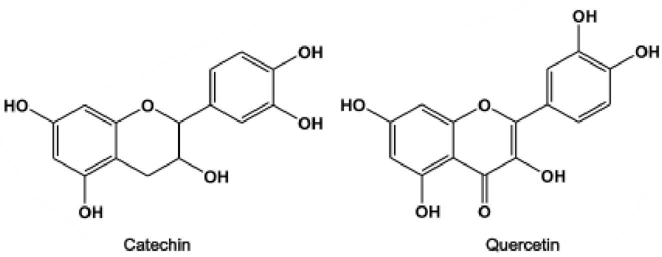
		n-hexadecanoic acid	
		α-terpinyl acetate	
		Verticiol	

Table 2. Quantitative phytochemical screening of *Gardenia jasminoides* J. Ellis.

No.	Plant Parts	Compound	Amount of substance contained (mg/g)	Reference
1.	Leaf	Alkaloid	2.81 ± 0.31	[21]
		Flavonoid	2.77 ± 0.20	
		Phenol	1.91 ± 0.24	
		Tannins	1.64 ± 0.13	
		Total phenolic	190.97	[10]
		Gallic acid	9.06 (mg/100 g)	
		Catechin	141.02 (mg/100 g)	
		Rutin hydrate	72.06 (mg/100 g)	
		Quercetin	19.06 (mg/100 g)	
		Total phenolic	3.69 ± 0.01	[22]
		Total flavonoids	7.03 ± 0.02	
2.	Fruits	Crocin	8.41	[12]
		Geniposide	109.0	
		Total phenolic	24.97 mg CAE/g	
		Geniposide	56.2 mg/500 mg ekstrak	[23]
		Total phenolic	1.72 ±0.01	[22]
		Total flavonoids	3.24 ± 0.04	
3.	Branch	Total phenolic	1.79 ± 0.02	[22]
		Total flavonoids	3.26 ± 0.01	
4.	Flower	Total phenolic	1.38 ± 0.03	[22]
		Total flavonoids	1.69 ± 0.15	
5.	Stem	Total phenolic	1.28 ± 0.04	[22]
		Total flavonoids	2.05 ± 0.01	
6.	Roots	Total phenolic	0.97 ± 0.01	[22]
		Total flavonoids	1.82 ± 0.01	

Leaf of Gardenia

Initial phytochemical screening results from gardenia leaf extract showed the presence of flavonoid compounds, tannins, glycosides, steroids, and alkaloids. The polyphenolic compounds found were rutin hydrate, catechins, quercetin, and gallic acid (Table 1). The structure of polyphenolic compounds from gardenia leaf extract is shown in Figure 1. Since the presence of flavonoid and tannin polyphenolic compounds in the extract was identified, the total phenolic content was determined using the Folin-Ciocalteu method. The high total phenolic content concluded that the antioxidant activity of the gardenia leaf extract was also high. The Folin-Ciocalteu method was determined by mg gallic acid equivalent per gram using the equation obtained from the standard gallic acid calibration curve[10]. The chemical content is quantitatively presented in Table 2. From the phenolic compounds found, catechins and rutin were the hydrates with the highest content. Flavonoids are secondary metabolites that are widely distributed in plants. It belongs to the group of polyphenolic compounds with known properties such as free radical inhibitors and anti-inflammatory properties.



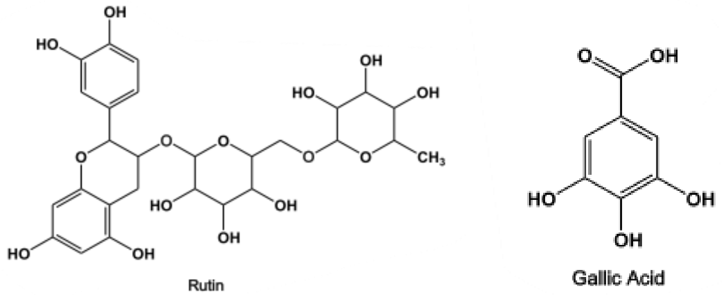
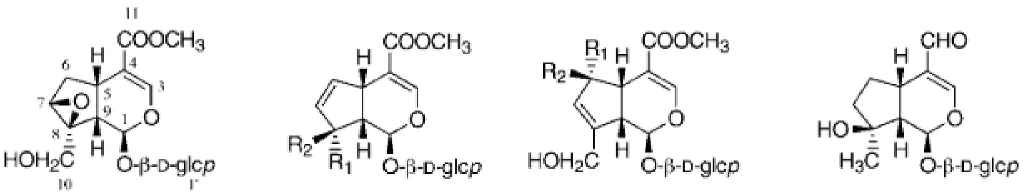


Figure 1. Chemical structure of polyphenolic compounds from gardenia leaf extract[10].

Other compounds found in gardenia leaves are iridoid glycosides. This group of monoterpene lactone compounds is most often found in plants that are bound to sugars as glycosides. Machida et. al. identified two new iridoid glycosides **(1, 2)** from the leaves of the *Gardenia jasminoides* plant isolated together with six iridoids **(3-8)** and three artifacts **(9-11)** from the gardenia plant (Table 1).

From the structural analysis, it was concluded that compounds **9**, **10**, and **11** may be artifacts formed from compounds **3**, **5**, and **6** during the extraction and isolation processes[11]. Iridoid glycosides that have been identified are known as monotropein methyl ester (=galioside, **3**), gardenoside **(4)**, deacetylasperulosidic acid methyl ester **(5)**, scandoside methyl ester **(6)**, geniposide **(7)**, and ixoroside **(8)**. Sample data are presented by comparison of various spectral and chemical data with the literature that has been reported. Reported biosynthetic sequences of these compounds are **7→5→3** and **7→6→4**. And compound **7** is the biosynthetic precursor of compounds **3** and **4**[11].

The following are characteristics of the two iridoids**(1, 2)** glycosides found. Compound **1** based on the identification of the structure named 7β, 8β-epoxy-8α-dihydrogeniposide **(1)** was obtained in the form of an amorphous powder, isolated from natural sources for the first time, although some acetate derivatives of this compound have been synthesized from compound **7**. Compound **2** with the name 8-epiapodantheroside **(2)** was obtained as an amorphous powder. The artifacts found were 8-O-methylmonotropein methyl ester **(9)**, 6-O-methyldeacetylasperulosidic acid methyl ester **(10)**, and 6-O-methylscandoside methyl ester **(11)**. The chemical structure of compounds **1-11** isolated from gardenia leaves is shown in Figure 2.



1

2: R_1=CH_2OH, R_2=H
3: R_1=OH, R_2=CH_2OH
4: R_1=CH_2OH, R_2=OH
9: R_1=OCH_3, R_2=CH_2OH

5: R_1=OH, R_2=H
6: R_1=H, R_2=OH
7: R_1=R_2=H
10: R_1=OCH_3, R_2=H
11: R_1=H, R_2=OCH_3

8

Figure 2. Chemical structure of compounds 1-11 isolated from gardenia leaves[11].

Fruit of Gardenia

Research on compounds in gardenia fruit has been carried out by many researchers as shown in Table 1. A study in 2013 found iridoid glycosides **(1-10)** and pyronane monoterpenoids **(11-20)**[16,24]. Among the 20 compounds that have been discovered, compounds **11** and **18** were obtained from this species for the first time[12]. The structure of compounds **1-20** isolated from gardenia fruit is shown in Figure 3.

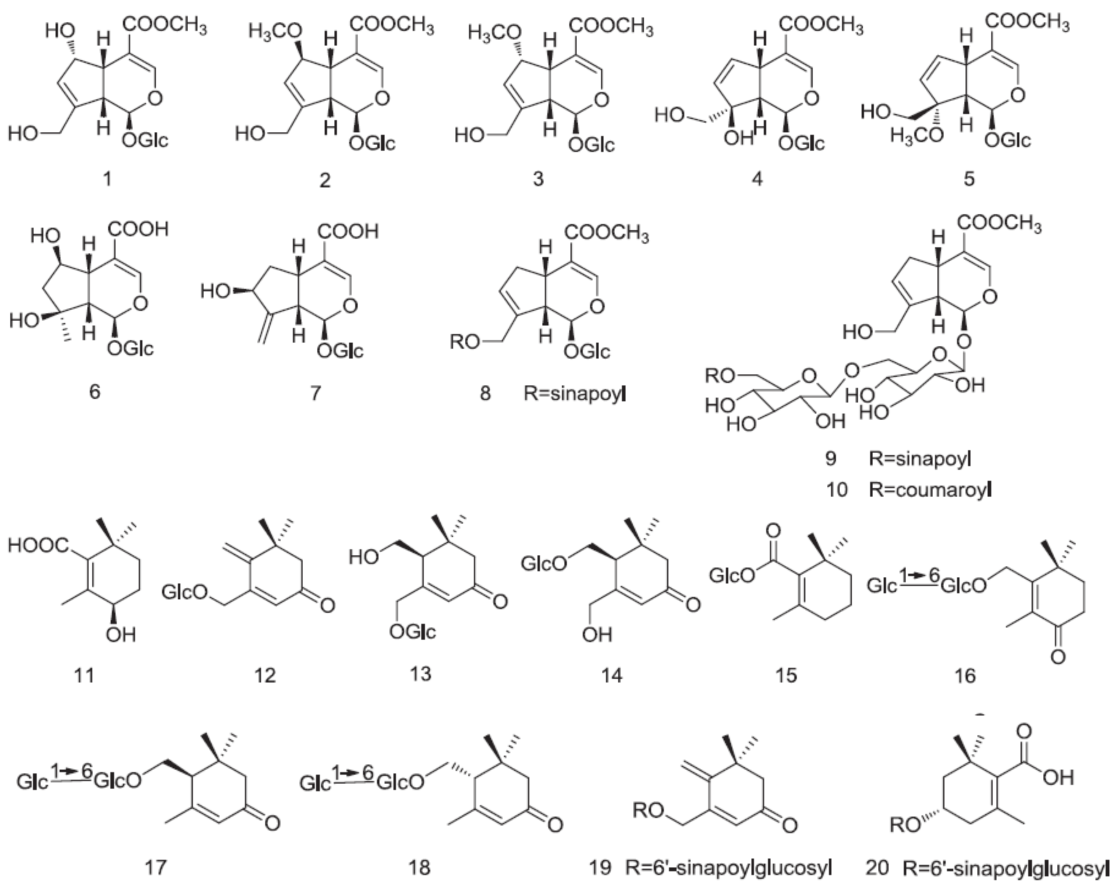


Figure 3. Chemical structure of compounds **1-20** isolated from gardenia fruit[16].

In another study in 2019, a group of iridoid glycosides was proposed (geniposide, geniposidic acid, secoxyloganin, gardenoside, genipin 1-gentiobioside, scandoside methyl ester, and shanzhiside), phenylpropanoid acid (chlorogenic acid, cryptochlorogenic acid, neochlorogenic acid, isochlorogenic acid A, isochlorogenic acid B, isochlorogenic acid C, and caffeic acid), and carotenoids (crocin-1 and crocin-2) (Table 1). The chemical structure of iridoid glycosides, phenylpropanoid acid, and carotenoids in gardenia fruit parts is shown in Figure 4.

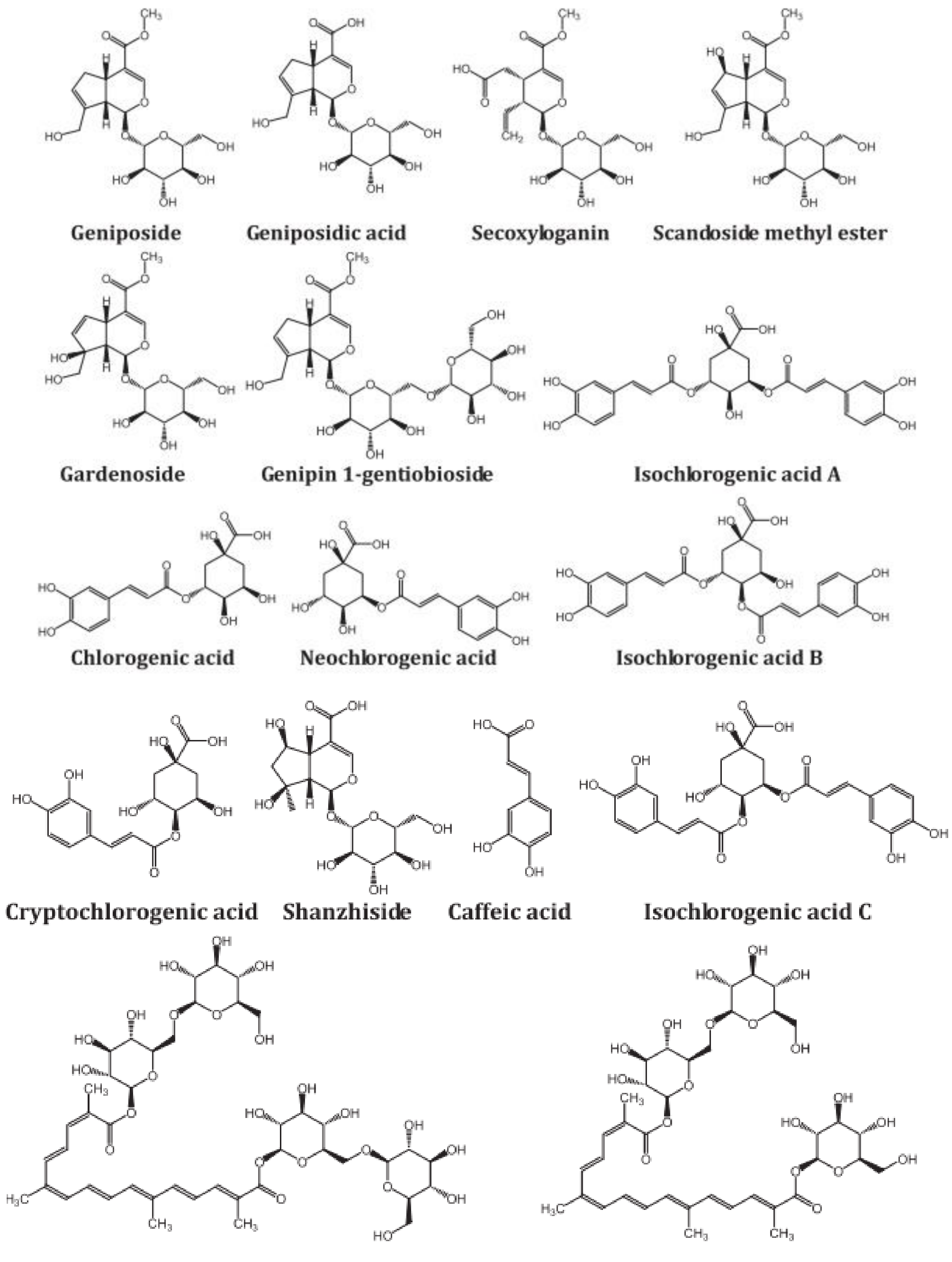


Figure 4. Chemical structure of iridoid glycoside, phenylpropanoid acid, and carotenoid compounds in gardenia fruit[13].

Research by Yang et. al (2009) carried out optimization of gardenia fruit extraction with the surface response methodology (RSM) which can optimize the extraction parameters of crocin, geniposide, and total phenolic compounds from the gardenia fruit. With this method, the production process, especially for factories, gets optimal results. The optimal extraction parameters obtained were the amount of ethanol 51.3%, extraction temperature 70.4ºC and time 28.6 minutes[12].

Gardenia fruit has been sold by various production companies in China. Research by Bergonzi et. al (2012) identified and quantified the content of gardenia fruit, namely iridoids, caffeoylquinic acid derivatives, and crocin using the HPLC method with five samples of gardenia fruit herbs sold from various production companies[25]. From the results of the analysis, it was found that the iridoid content was a geniposide in each sample. There were no qualitative differences in content but quantitative differences in constituents were found. This is due to different sources or different processing methods. The variability in the content of these samples can be attributed to the geographic region of origin rather than the method of processing the plant material[25]. In addition, the level of maturity of gardenia fruit has nothing to do with the content of crocin and geniposide which is already present in the fruit and does not affect its antioxidant activity[25].

Research by several experts in 2001-2004 found the main compound of *Gardenia jasminoides* J. Ellis fruit, namely geniposide, which is an iridoid glycoside and shows a chemopreventive effect on early acute liver damage caused by aflatoxin B1 and also has detoxifying, anti-tumor, and anti-tumor effects. and anti-thrombotic activity[23]. In previous studies, to obtain geniposide compounds from the fruit of *Gardenia jasminoides* J. Ellis used conventional methods that required multiple column chromatography (CC) and high-performance liquid chromatography (HPLC). Therefore, it is necessary to develop a simple and efficient method for the isolation of geniposide compounds from the fruit of *Gardenia jasminoides* J. Ellis. Using centrifugal partition chromatography (CPC) is one of them. This type of chromatography does not undergo irreversible sample adsorption and has been widely used for the separation of natural product metabolites[23]. In addition to using the centrifugal partition chromatography (CPC) method, the first-step analysis with HPLC at 254 nm was needed to see the compound content of the gardenia fruit extract which contains several compounds including geniposide as the main element. After that, it was continued with the centrifugal partition chromatography (CPC) method with a two-phase solvent system because the nature of the geniposide compound was soluble in aqueous solvents. Samples of 500 mg of gardenia fruit extract were separated by CPC over 100 minutes and the composition of the collected fractions was determined by HPLC. As a result, 56.2 mg of geniposide was isolated from 500 mg of extract. From the results obtained, the centrifugal partition chromatography (CPC) method can be applied to purify geniposide compounds and evaluate the pharmacological effects of gardenia fruit[23].

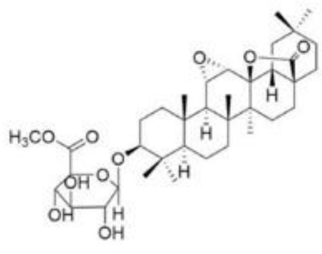
Research by Wang et. al (2014) used the method of separation and purification of iridoid glycosides and crocetin derivatives using the High-speed Countercurrent Chromatography (HSCCC) method as a lower cost process[15]. Iridoid glycosides and crocetin derivatives are the two main active constituents in gardenia fruit which have been widely studied and published[11,23,26,27]. The HSCCC method for the separation and chemical purification of gardenias has been previously reported by Zhou et al. al. 2005 by producing a geniposide compound. Then research by Liang et. al 2014 reported the isolation and purification of three compounds, namely geniposide, crocin-1, and geniposide acid[15].

The first treatment of gardenia fruit was extraction to fractionation. Dried gardenia fruit was ground into a coarse powder and then extracted with ethanol-water by cold percolation method. After being fractionated by column chromatography, several solvents with a certain ratio were used to purify the compound. And do isolation with HSCCC. After isolation, gardenoside, 6-hydroxy geniposide, and geniposide acid were found in fraction A. In fraction B only geniposide was obtained. Meanwhile, in fraction C, crocin-1, crocin-2, crocin-3, and crocin-4 were obtained. The purity level of the compounds obtained was an average of 94.9% with the help of UPLC analysis. These results indicate that iridoid glycosides and crocetin derivatives in gardenia can be obtained efficiently from the extract using HSCCC[15].

From several studies, experts in isolating the chemical compounds present in the gardenia fruit can be done by any method such as the isolation method in general, namely chromatography. Learning from existing research, researchers always use ethanol or methanol as a solvent to extract it. This is because ethanol and methanol are universal organic solvents used in extraction activities that can filter secondary metabolites such as glycoside compounds. Because the majority of secondary metabolites are semi-polar.

Root of Gardenia

In southern China, the use of gardenia root as a herbal medicine for the treatment of hepatitis and nephritis has been widely used. Until now the literature that discusses the chemical content of the roots is still very little. From a previous literature study in 1986, it was only known that gardenia roots contained iridoids, oleanolic acetic acid, and olarstigmasterol. Then in 2012 Wang et al. reported the results of their research by finding 10 triterpenoid compounds **(1-10)** from the ethanolic extract of gardenia roots (Table 1). Compounds **1-3** are triterpene compounds that have just been reported and named Gardenicide A, Gardenicide B, and Gardenicide C[17]. The chemical structure of compounds **1-10** isolated from gardenia root is shown in Figure 5.



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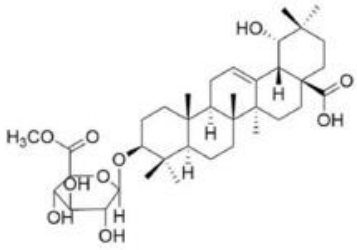

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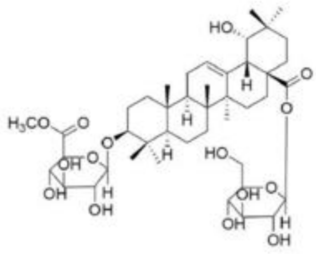

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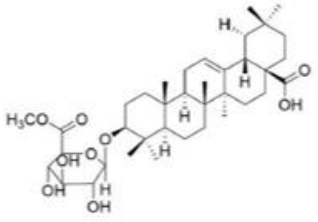

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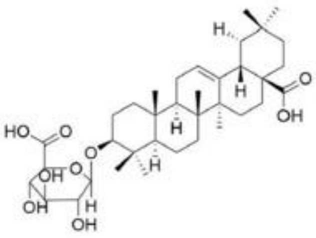

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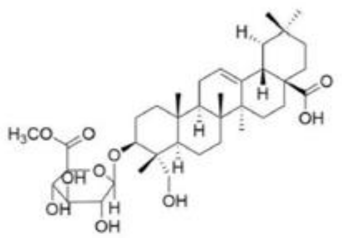

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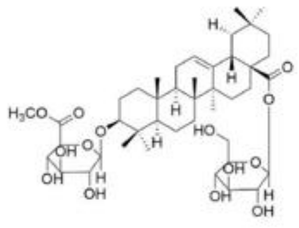

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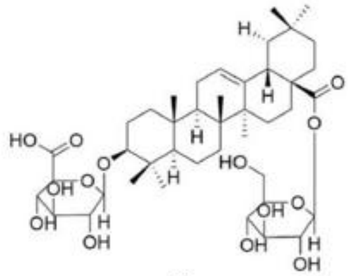

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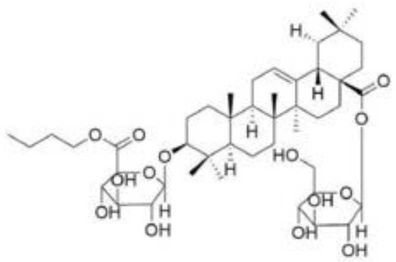

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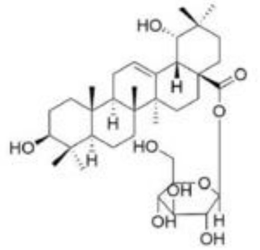
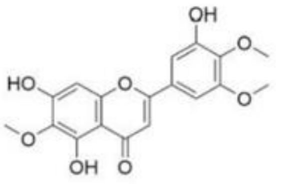


(10)

Figure 5. Chemical structure of compounds **1-10** isolated from gardenia roots[17].

Flower of Gardenia

Publications about the chemical content of the gardenia flower are quite small compared to the gardenia fruit. From these studies, the chemical content of the gardenia flower is very diverse, starting from carotenoid compounds, iridoid glycosides, flavonoids, triterpenoids, to organic acids and their derivatives. Table 1 has shown the chemical content of gardenia flowers from several references. In 2013 Song et. al. published their research with the results of 15 compounds from gardenia flowers. With details, compounds **1**, **2**, **3**, and **4** are groups of flavonoid compounds, compounds **5**, **6**, **7**, **13**, **14**, and **15** are groups of organic acid compounds and their derivatives, compounds **8** and **9** are anthraquinones, compounds**10** are carotenoids, and compound **11** and **12** from the triterpene group. The chemical structure of compounds **1-15** isolated from gardenia flowers is shown in Figure 6. The compound quercetin **(4)** is the same flavonoid found in gardenia leaves. The compound crocin-1 **(10)** is a compound that has been known as a yellow pigment in the food coloring industry. This compound is also found in the fruit of the gardenia plant. Crocin belongs to a group of carotenoid compounds that are water-soluble.



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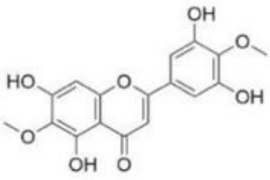

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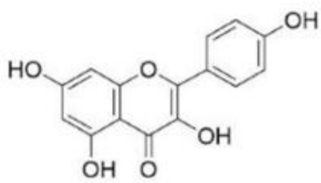

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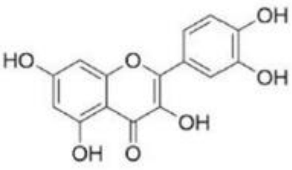

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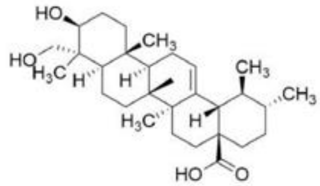

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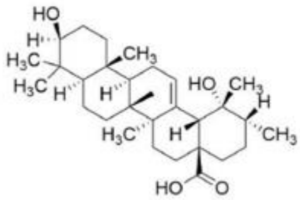

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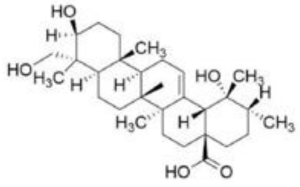

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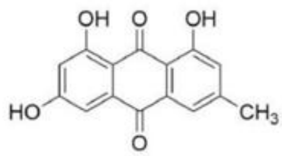

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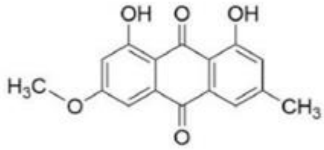

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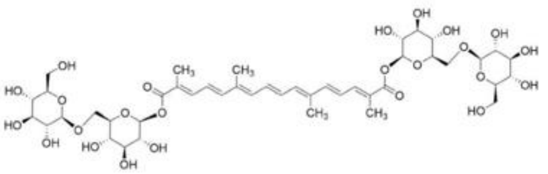

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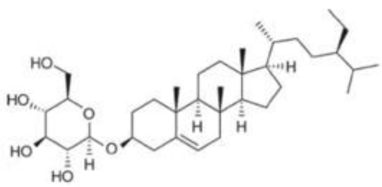

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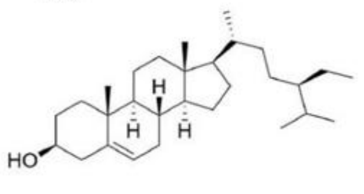

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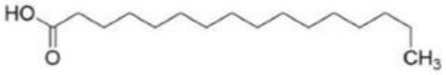

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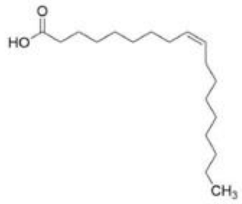

(15)

Figure 6. Chemical structure of compounds **1-15** isolated from gardenia flowers[18].

Then in 2017, the publication by Zhang et. al. found four new iridoid glycosides isolated from gardenia flowers and a new phenylpropanoid glucoside compound, as well as 16 other compounds that have been identified by other studies. The five newly discovered compounds are shown in Table 1 and Figure 7. From the 16 known compounds, 12 of the iridoid glycosides are geniposide **(6)**, gardenoside B **(7)**, 6β-hydroxygeniposide **(8)**,6α-hydroxygenipine **(9)**, 6α-hydroxygeniposide **(10)**, 7β,8β-epoxy-8α-dihydrogeniposide **(11)**, genipin 1-O-β-D-isomalltoside **(12)**, genipin-1,10-di-O-β-D-glucopyranoside **(13)**, 6α-butoxygeniposide **(14)**, garjasmine **(15)**, 6β-ethoxygeniposide **(16)**, and 2'-O-trans-p-coumaroylgardoside **(17)**. Two phenolic acid compounds are 4,5-diferuloylquinic acid **(18)** and niacin **(19)**. As well as flavonoid compounds 5,7,3',5'-tetrahydroxy-4'-methoxyflavone **(20)**, and 5,7,5'-trihydroxy-6,3,4'-trimethoxyflavone **(21)**.

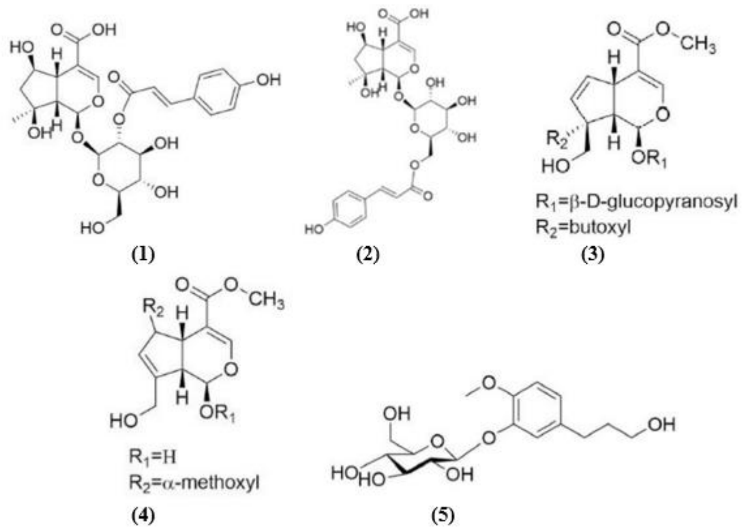

Figure 7. Chemical structure of compounds **1-5** isolated from gardenia flowers[19].

Another ingredient is an essential oil from gardenia flowers reported by Zhang et. al. 2020. By finding 16 compounds with a relative content of more than 1% consisting of a group of terpene compounds, alcohols, and esters were extracted by hydrodistillation and their compound contents were analyzed by GC-MS (Table 1). The main components of gardenia flower essential oil are linalool (34.7%), α-farnesene (10.2%), α-terpineol (6.3%), geraniol (5.8%), and cembrene A (5.8%). The chemical structure of the main components of gardenia flower essential oil is shown in Figure 8.

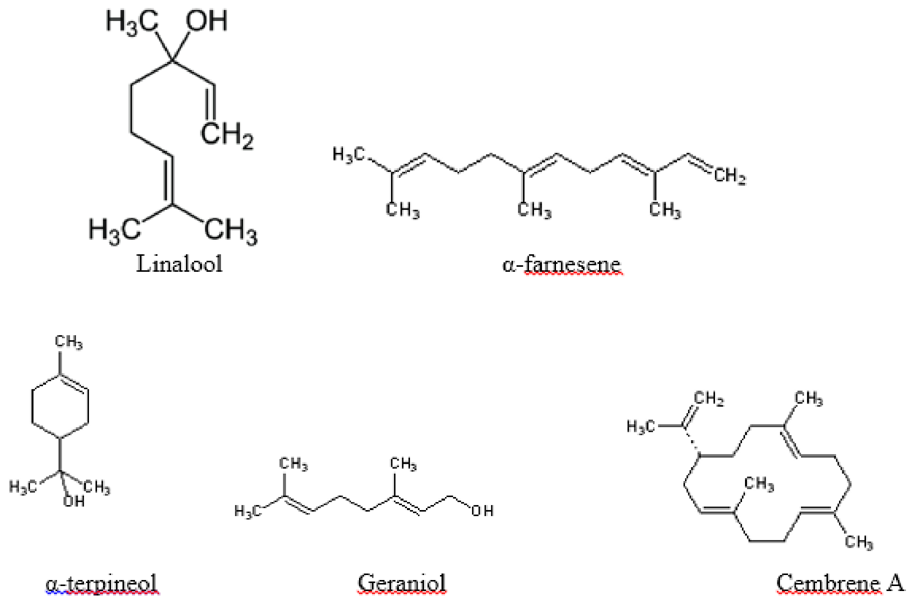

Figure 8. Chemical structure of the main components of gardenia flower essential oil.

Antioxidant Activity

The many contents of gardenia are flavonoids and polyphenols which are known to provide benefits as antioxidants that can capture free radicals. Free radicals are compounds that have one or more unpaired electrons and are normally generated in cellular metabolism. Reactive oxygen molecules and reactive nitrogen molecules are examples of reactive free radicals that can cause disintegration of cell membranes, damage to membrane proteins, and DNA mutations, which in turn can initiate and multiply the development of many diseases, such as cancer, liver disease, and cardiovascular disease[28,29].

The mechanism of cell damage by free radicals in three ways, namely[30]:

1. Peroxidation of lipid components of cell membranes and cytosol by reducing fatty acids (autocatalysis) which causes damage to cell membranes and organelles.
2. Damaging DNA can lead to DNA mutations and even cell death.

3. Modification of oxidized proteins due to the formation of *cross-linking* proteins, through sulfhydryl mediators on several labile amino acids such as cysteine, methionine, lysine, and histidine.

One way to avoid the negative effects of free radicals is with antioxidants. Naturally, the body has an antioxidant system that can counteract free radicals, either through enzymatic or non-enzymatic processes. Antioxidants can be defined as electron-donating compounds needed by free radicals in stabilizing themselves, and can also stop the formation of free radicals[31,33]. Antioxidants can neutralize free radicals and can prevent harmful body biological changes due to excessive oxidation[31]. The mammalian body has antioxidant defense mechanisms such as catalase, superoxide dismutase, glutathione peroxidase enzyme, and antioxidant nutrients, namely vitamin E, and ascorbic acid which resist the destructive properties of reactive oxygen species. Exposure to certain chemicals and contaminants can lead to increased generation of free radicals in the body beyond its capacity to control them, and ultimately cause permanent damage to tissues and cellular components[10].

Natural antioxidants are found in all parts of higher plants, such as wood, stems, leaves, fruits, roots, flowers, and pollen. These compounds are usually phenolic or polyphenolic compounds[32]. Antioxidants in higher plants have been tested in vitro, able to provide protection from damage caused by oxidation, inhibit and bind free radicals, and reactive oxygen. Singlet oxygen is strongly bound by carotenes, especially -carotene. Phenolic components act as antioxidants depending on the redox value of the hydroxyl group, with the mechanism of reducing, hydrogen donor and oxygen binding[33].

Leaf of Gardenia

Based on research by Uddin et. al published in 2014 conducted a study on the antioxidant activity of gardenia leaves using a thick extract of gardenia leaves obtained from soxhlet extraction with 95% methanol. From the phytochemical screening of gardenia leaf extract, it was found that gardenia leaf extract contains polyphenol and flavonoid compounds which are compounds that provide antioxidant properties. The determination of antioxidant activity used by researchers is the DPPH method. DPPH is a relatively stable nitrogen-centered free radical that can readily accept an electron or hydrogen radical to form a more stable diamagnetic molecule. The DPPH antioxidant assay was developed based on the ability of 1,1-diphenyl-2-picryl-hydrazyl (DPPH), a stable, odd-electron-containing free radical, which is responsible for the purple color of DPPH in an alcoholic solution and the color intensity can be measured at an absorbance of 515 nm. With the ability to decolorize in the presence of antioxidants so that it can be measured quantitatively from the change in absorbance[10].

In addition to looking at the antioxidant activity with DPPH, the researchers also saw it from its reducing power. This method is attributed to the presence of reductants whose mechanism exerts antioxidant action by breaking free radical chains by donating hydrogen atoms. It was also reported that reductants can react with certain peroxide precursors, thereby preventing the formation of peroxides[10]. From the method of determining the antioxidant activity of gardenia leaf extract, both methods showed an increase in antioxidant activity as the concentration of the sample extract increased compared to ascorbic acid. These results were due to the high total amount of phenolic compounds in the methanolic extract of gardenia leaves, which was 190.97 ± 10.37 mg gallic acid equivalent[10].

Fruit of Gardenia

Gardecin compounds from gardenia fruit were separated and purified by the HSCCC (High-speed Counter-current Chromatography) method conducted by Chen et. al (2005). Then, the antioxidant activity was determined using the anti-hemolytic activity method, lipid peroxidation inhibition, and the phosphomolybdenum method. With the lipid peroxidation inhibition method, it showed significantly stronger activity than the crocin-1 compound. In addition, gardecin showed significantly greater potency (126.3 mg α-tocopherol/g) than crocin-1 (79.2 mg α-tocopherol/g). Antioxidant activity was also compared with crocin-1 having strong antioxidant activity. These results indicate that the HSCCC method can be used efficiently to obtain compounds[34]. Gardecin compounds were also characterized by spectrometric techniques, especially NMR. Antioxidant activity compared to other crocins, surprisingly the free radical DPPH ability of gardecin was higher and quite stable than other crocins. Water-soluble compounds crocin and crocetin aglycones, a group of carotenoid compounds, have been isolated from gardenia and saffron, and their pharmacological effects have been investigated by experts, including protection from cardiovascular disease[35–37] and inhibition of tumor cell proliferation[38]. From the various pharmacological effects that have been disclosed, it is hypothesized that antioxidant compounds are behind all the pharmacological effects of carotenoids. Research by Pham et. al published in 2000 reported that crocin-1 exhibited strong antioxidant activity by the thiocyanate method[39].

Research by Chen et. al (2008) revealed that crocin is the main pigment in gardenia fruit but is not a major contributor to antioxidant activity. There was no correlation between total crocin content and antioxidant function. But what is associated with the antioxidant activity is the total content of phenolic compounds[40]. The compounds genipine, chlorogenic acid, rutin, and ursolic acid have been found in the ethanolic extract of gardenia fruit[14]. Among these compounds, genipin and ursolic acid showed antioxidant activity. Genipin is an aglycone compound of the iridoid glycoside geniposide. Research conducted by Lee et. al. published in 2009 to examine the effect of gardenia fruit extract as antigastritis showed genipin and ursolic acid inhibited significant gastric lesions which concurrently had antigastritis activity related to the antioxidant activity, acid neutralizing capacity, and antibacterial of H. pylori. The antioxidant activity of gardenia ethanol extract described by the DPPH method showed significant free radical scavenging activity[41].

Research by He et. al (2010) identified antioxidant compounds in gardenia fruit extracts. Nine compounds were identified, namely (3-caffeoylquinic acid, 4-caffeoylquinic acid, 5-caffeoylquinic acid, 3,4-dicaffeoylquinic acid, 3,5-dicaffeoylquinic acid, 4,5-dicaffeoylquinic acid, quercetin-3-rutinoside, crocetin di(β-gentiobiosyl) ester, and geniposide)[42]. Among these compounds, caffeoylquinic acid, dicaffeoylquinic acid, and 4-sinapoyl-5-caffeoylquinic acid were the dominant free radical scavengers in gardenia fruit extracts, and their antioxidant capacity was determined by Trolox equivalent antioxidant capacities (TEAC)[42]. And the 3,5-dicaffeoylquinic acid was the most powerful antioxidant with a TEAC value of 1.1 mM. In contrast, crocetin di(β-gentiobiosyl) ester and geniposide showed little scavenging activity by the ABTS method. This proves the research of Chen et. al. 2008 who found that crocin was not a major contributor to antioxidant activity. There was no correlation between total crocin content and antioxidant function. But what is associated with the antioxidant activity is the total content of phenolic compounds[40,42].

From previous research, it has been known that gardenia fruit contains essential oils. He et. al (2010) analyzed the oil content of gardenia fruit using the HPLC method. And it was found that gardenia fruit oil predominantly contains α-tocopherol. Determination of antioxidant activity is seen by on-line HPLC combined with DPPH or ABTS tests. Of the significant free radical scavenging activities, they were identified as α-tocopherol and γ-tocopherol based on the UV spectrum and retention time of standard compounds. Meanwhile, β-tocopherol showed weak antioxidant activity in the sample. From the correlation analysis of DPPH radical scavenging activity with total tocopherol content, it shows a good correlation coefficient, it is concluded that tocopherol is the main antioxidant in gardenia fruit oil[43]. Research by Debnath et. al. (2011) reported that gardenia fruit water and ethanol extracts showed strong reducing power. The aqueous extract had higher antioxidant activity than the ethanol extract. The high antioxidant activity is related to the phenolic and flavonoid content found in plants[44].

Research by Wang et. al. published in 2019 reported the antioxidant activity of various parts of *Gardenia jasminoides* namely roots, stems, leaves, branches, flowers, and fruits originating from China. To ensure the presence of antioxidants, the total phenolic compounds and total flavonoids were determined. This compound has been shown to act as an antioxidant[22]. The extraction process of each part of the *Gardenia jasminoides*plant was carried out using an ultrasonic extraction approach using 80% ethanol at room temperature, then centrifuged. The supernatant formed or the upper part of the results of this centrifugation is taken as a sample for testing with reagents. Determination of antioxidant activity in various parts of *Gardenia jasminoides* was carried out using the DPPH method[22]. To determine the total polyphenol content, the Folin-Ciocalteu method was used with a gallic acid standard measured absorbance at a wavelength of 764 nm. As well as the determination of the total flavonoid content was carried out with the reagent $NaNO_2$-$Al(NO_3)_3$ with the standard fagopyrol measured absorbance at a wavelength of 508 nm[22]. The radical scavenging activity of DPPH (IC_{50}) from leaf ethanol extract was the highest, followed by twigs, fruit, flowers, stems, and roots. For the polyphenol content, the extract from the leaves had the highest polyphenol content, followed by twigs, fruit, flowers, stems, and roots. Meanwhile, the total flavonoid content also showed that extracts from the leaves had the highest content, followed by twigs, fruit, stems, roots, and flowers[22]. From all the tests carried out, the leaf extract of *Gardenia jasminoides* always contains the highest antioxidant activity, polyphenols, and flavonoids, which are 2-3 times more than the stems, branches, flowers, and fruit, and 4 times more than the roots of the Gardenia plant. This is very relevant to the utilization of the leaves of the *Gardenia jasminoides* plant which is most widely used by the public as herbal medicine because it is proven to have a healing effect because of its content.

IV. Conclusions

Gardenia jasminoides is generally used as a traditional medicine to reduce fever or fever and digestive disorders. The yellow pigment in gardenia fruit has long been used as a natural dye in the food and textile industries in China. Several studies have reported that the compounds contained in gardenia plants provide pharmacological effects, including anti hepatic, choleratic, diuretic, laxative, detoxifying, chemopreventive, and anti-tumor activities. The literature review shows that phenolic compounds, flavonoids, iridoid glycosides, monoterpenoids, triterpenoids, carotenoids, organic acids, and their derivatives have been isolated and identified from all parts of the gardenia plant. The number of compounds identified is about 104 compounds. Among them, a group of iridoid glycoside compounds such as geniposide is considered to be the bioactive ingredient that is characteristic of gardenia plants. The phenolic compounds contained in gardenia plants have the potential as antioxidants. With the large value of traditional uses that have been obtained, a comprehensive analysis of the content of the gardenia plant is urgently needed, especially its antioxidant activity which is the source of all neutralizing degenerative diseases that many humans suffer in the world. Until now, gardenia products have only been produced by factories in China in the form of herbal medicines. Therefore, in-depth analysis is still needed to obtain stable and standardized compounds that are formulated into dosage forms that are easy to obtain and use.

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