Phytochemical Study on *Rhodiola kirilowii*

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Abstract of thesis entitled:
Phytochemical Study on *Rhodiola kirilowii*
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Abstract

Radix Et Rhizoma Rhodiolae is a traditional Chinese medicine used in Xin Jiang and Tibetan region of China. It is regarded as an adaptogen. The anti-oxidizing effect of this drug has been reported and its potential usage in treating acute mountain sickness was suggested. Besides, biological and clinical experiments have been carried out to investigate the anti-tumor effect and the protective effect of this drug on the circulatory system. However, the phytochemical information of this herb is not well documented. In this research, phytochemical study was conducted in *Rhodiola kirilowii,* one of the five species of Radix Et Rhizoma Rhodiolae.

Twelve compounds were isolated from *Rhodiola kirilowii*. Among these twelve compounds, ten compounds, namely *trans*-hydroxycinnamic acid, sacranoside B, geranyl-β-glucopyranoside, neryl-β-glucopyranoside, rhodiolgin, rhodiooctanoside, gallic acid, (-)-epigallocatechin 3-gallate, isolariciresinol-9-O-β-glucopyranoside, and hexyl β-glucopyranoside, were the

first time isolated and purified from this species, and geranyl-β-glucopyranoside and neryl-β-glucopyranoside were the first time being found in this genus.

論文題名:
*狹葉紅景天*的植物化學研究
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摘　　要

紅景天是新疆、西藏地區常用中藥，被列爲適應原 （adaptogen）。在學術研究層面上，研究人員對紅景天的抗氧化功能作了出廣泛的研究，並積極將紅景天發展爲治療高山症的藥物。此外，科學家在紅景天的抗癌及保護血管等功用的課題上亦作出了不少生物性和臨床性的實驗，希望能更了解這種中藥的作用。然而，有關紅景天植物化學方面的研究卻很少。

狹葉紅景天（*Rhodiola kirilowii*）是五種藥用紅景天品種之一。本篇研究論文，著重於狹葉紅景天的植物化學研究。整個研究共分離及鑒定出十二個化合物，分別是 *β*-sitosterol，tyrosol，*trans*-hydroxycinnamic acid，sacranoside B，geranyl-*β*-glucopyranoside，neryl-*β*-glucopyranoside，rhodiolgin，rhodiooctanoside，gallic acid，(-)-epigallocatechin 3-gallate，isolariciresinol-9-O-*β*-glucopyranoside 及 hexyl *β*-glucopyranoside。其中，*trans*-hydroxycinnamic acid，geranyl-*β*-glucopyranoside，neryl--glucopyranoside，rhodiolgin，rhodiooctanoside，gallic acid，(-)-epigallocatechin gallate，(+)-isolariciresinol-9-O-*β*-glucopyranoside 及

hexyl *β*-glucopyranoside 是第一次從狹葉紅景天中發現，而 geranyl-*β*-glucopyranoside 及 neryl-*β*-glucopyranoside 則是第一次從紅景天屬植物中發現。

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$[\alpha]_D$	specific optical rotation
d	Doublet
dd	double-doublet
DEPT C-13	distortionless enhancement by polarization transfer
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
g	gram
HPLC	high performance liquid chromatography
Hz	hertz
IR	infrared
J	coupling constant
kg	kilogram
m	multiplet
MS	mass spectrometry
MW	Molecular weight
NMR	Nuclear magnetic resonance
RP	Reversed phase
s	singlet
t	triplet
TLC	Thin layer chromatography
UV	Ultra-violet

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Chapter 1 Introduction

Radix Et Rhizoma Rhodiolae is a traditional Chinese medicine used in Xin Jiang and Tibetan region of China. It belongs to the *Rhodiola L.* genus of the Crassulaceae family. There are about 90 species in this genus, of which over 70 species can be found in China [1]. Five species are used for medicinal purposes, they are, *R. kirilowii*, *R. dumulos*, *R. algida*, *R. yunnanensis* and *R. henryi* [2].

Species in the *Rhodiola L.* genus are perennial herbaceous plants, growing at altitude between 2000-5000 meters. It can be found in the Northern, Northeastern, Northwestern and Southwestern parts of China. The roots and rhizomes are used for medical treatments. Chinese Medical publications [3] stated that this herb has protective effect on lungs, on blood vessels, against influenza and reduce edema as well as tissue swelling.

In Qing Dynasty, Radix Et Rhizoma Rhodiolae was used as a reward to generals and soldiers after a victory. Nowadays, it is used as a common drug to treat and prevent the development of Acute Mountain Sickness [4, 5]. Besides, clinical trials and animal experiments have been done on the anti-oxidative effect and anti-hypoxia effect of the drug [6, 7]. In addition, researches are also carried out on anti-aging, anti-fatigue and anti-cancer

effects of the drug [8].

Although pharmacological effects of this drug have been studied extensively, the phytochemistry of the plant is not clearly understood. In order to elucidate the action mechanism of an herbal drug, it is necessary to understand the chemical composition. *Rhodiola kirilowii,* although belongs to one of the five medicinal herbs, is seldomly investigated on its phytochemical properties. In this respect, this project aims to isolate the constituents and determine their structures from *Rhodiola kirilowii*, to provide chemical information for the further pharmacological studies of the species.

Rhodiola kirilowii is distributed in the mountain slopes and underwood regions at altitude between 3100-5600 meters. It can be found in Tibet, Shan Xi, He Bei, Yunnan and Si Chuan provinces. Both the root and rhizome parts of the herb are used for medical purposes. Traditional Chinese medicine publications stated that it can nourish the lung, relieve coughing and swelling of tissue. It is used in treating pneumonia, bronchitis and tuberculosis [9].

1.1 Chemical Constituents of *Rhodiola* Genus

Phytochemical studies of the *Rhodiola* genus started in late 1970s, mainly by Russian researchers [10]. Until early 1990s, Chinese researchers started the phytochemical study on this genus [11]. There are about 20 species being investigated, in which most of the researches are focused on *Rhodiola rosea* [12-15]. In this project, eleven species are chosen for review on the phytochemical study. They are *R. atuntsuensis, R. fastigiata, R. pamiera, R. rosea, R. sachalinensis, R. dumulosa, R. henryi, R. kirilowii, R. algida, R. yunnanensis* and *R. crenulata.*

Compounds isolated from the genus can be divided into several categories, namely, phenylethyl derivatives, phenylpropanoids, phenolic derivatives, flavonoids, monoterpenoids and triterpenes.

1.1.1 Phenylethyl Derivatives

Phenylethyl derivatives are compounds bearing a phenyl-4-ethyl group (Figure 1-1). Up to now, four compounds are found as phenylethyl derivatives in the eleven species of *Rhodiola*, which are salidroside, tyrosol, 6-O-galloylsalidroside and 2-phenylethyl β-glucopyranoside (Figure 1-2).

Tyrosol and salidroside are often considered the active ingredients of this drug. In *Rhodiola kirilowii*, the content of tyrosol is about 0.0048%, and that of salidroside is ranged between 0.003-0.5%, depending on the location of cultivation [16-18]. There are numerous studies being done on the anti-oxidizing effect of salidroside and tyrosol. Zhen *et. al.* [19] investigated the structural relationship of salidroside and its derivatives for their anti-oxidizing activity. Three factors were attributed to the anti-oxidizing activity of these derivatives: the length of carbon skeleton connecting to the aromatic ring; the positions of hydroxyl groups in the aromatic ring, and the types of sugar group connecting to the aglycone. Among them, the type of sugar group has the most pronounced effect on the anti-oxidizing power. Among galactose, glucose and mannose, galactose is the most effective sugar group, whereas mannose is the least effective.

Figure 1-1. Skeleton structure of phenylethyl derivatives

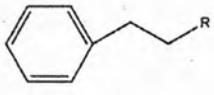
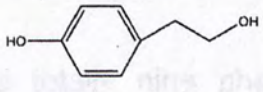


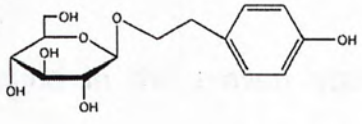
Table 1-1. Phenylethyl derivatives from *Rhodiola* species

Name	Plant source(s)	Ref.
Tyrosol	*R. atuntsuensis, R. fastigiata, R. rosea, R. sachalinensis, R. kirilowii, R. crenulata*	[20-25]
Salidroside	*R. atuntsuensis, R. fastigiata, R. rosea, R. sachalinensis, R. kirilowii, R. crenulata*	[20-25]
6-O-Galloylsalidroside	*R. sachalinensis*	[21]
2-Phenylethyl β-glucopyranoside	*R. sachalinensis*	[21]

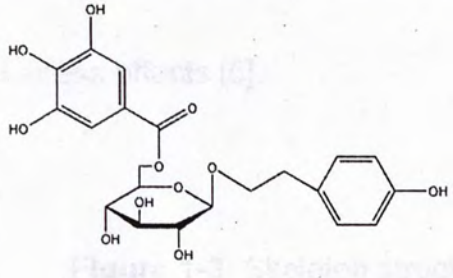
Figure 1-2. Phenylethyl derivatives isolated from *Rhodiola* species



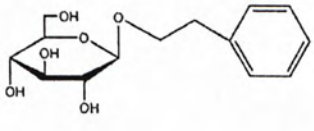
Tyrosol



Salidroside



6-O-Galloylsalidroside



2-Phenylethyl β-glucopyranoside

1.1.2 Phenylpropanoids

Phenylpropanoids are compounds possessing a phenyl-propanyl group (Figure 1-3). It is a group of plant-derived compounds that are synthesized from phenylalanine. Plants produce phenylpropanoids for a diversity of functions, such as to defense against herbivores and microbial attack; as structural components of cell wall, and as signaling molecules [26]. For example, cinnamic acid is synthesized from phenylalanine by phenylalanine ammonia lysase (PAL). It is then converted to a range of other organic acids and corresponding esters to attract pollinators.

There are totally nine phenylpropanoids found in the eleven species of *Rhodiola* (Figure 1-4). Rosarin and rosavin (rosavidine) are considered the active ingredients of the herb. Rosavin is reported to have anti-tumor and anti-stress effects [5].

Figure 1-3. Skeleton structure of phenylpropanoid compounds

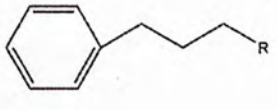
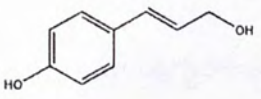


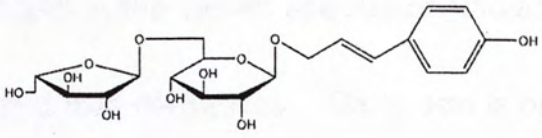
Table 1-2. Phenylpropanoids isolated from *Rhodiola* species

Name	Plant source(s)	Ref.
Cinnamyl alcohol	*R. rosea, R. kirilowii*	[23, 24]
β-(*E*)-cinnamyl-*O*-6'-*O*-*α*-arabinofuranosyl) –glucopyranoside	*R. sachalinensis*	[21]
trans-p-Hydroxycinnamic acid	*R. sachalinensis*	[21]
Dihydroconiferyl alcohol gamma-*O*-*β*- glucopyranoside	*R. fastigiata*	[22]
Rosarin	*R. rosea*	[27]
Rosavin (rosavidine)	*R. rosea, R. sachalinensis, R. kirilowii*	[24, 27, 28]
Rosin	*R. rosea, R. sachalinensis*	[21, 27]
Triandrin	*R. kirilowii*	[24]

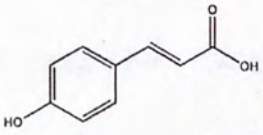
Figure 1-4. Phenylpropanoids isolated from *Rhodiola* species



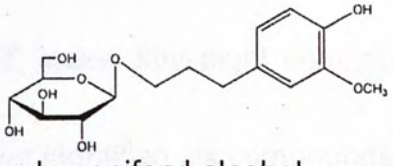
Cinnamyl alcohol



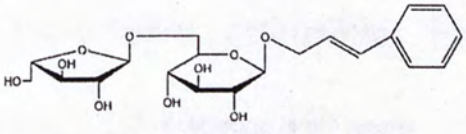
β-(*E*)-cinnamyl-*O*-(6'-*O*-*α*-arabinofuranosyl) - glucopyranoside



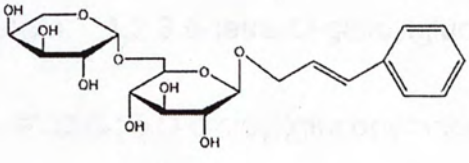
trans-*p*-Hydroxycinnamic acid



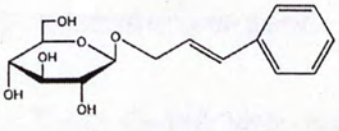
Dihydroconiferyl alcohol gamma-*O*-*β*-glucopyranoside



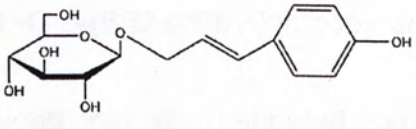
Rosarin



Rosavin (rosavidine)



Rosin



Triandrin

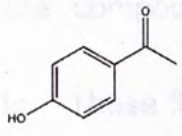
1.1.3 Phenolic Derivatives

There are nine phenolic derivatives found in the eleven species of *Rhodiola* (Figure 1-5). Among them, six are gallic acid derivatives. Gallic acid is one of the well known natural anti-oxidants. Gallic acid and its derivatives help protect against β-amyloid toxicity, the corresponding oxidative stress and the resulting apoptosis. Mook-Jung *et. al.* [29] tested fifty eight compounds on their protective effects against β-amyloid, and identified six compounds having significant protective effect, namely, 1,2,3,6-tetra-*O*-galloylglucose, (-)-epicatechin 3-*O*-gallate, (-)-epicatechin, 1,2,3,6-tetra-*O*-galloylglucose, *trans*-3,5,4'-trihydroxystilbene 4'-*O*-β-(2-*O*-galloyl)glucopyranoside, *trans*-3,5,4'-trihydroxystilbene 4'-*O*-β-(6-*O*-galloyl)glucopyranoside, *cis*-3,5,4'-trihydroxystilbene 4'-*O*-β-(6-*O*-galloyl)glucopyranoside. 1,2,3,6-Tetra-*O*-galloylglucose and (-)-epicatechin also showed significant anti-oxidative effect. This may indicate that natural products containing gallic acid and its derivatives would be good candidates for anti-oxidizing drug developments.

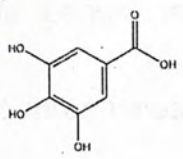
Table 1-3. Phenolic compounds isolated from *Rhodiola* species

Name	Plant source(s)	Ref.
p-Hydroxyacetophenone	*R. pamiera*	[30]
Gallic acid	*R. atuntsuensis, R. fastigiata, R. pamiera, R. rosea, R. sachalinensis, R. henryi, R. yunnanensis,R. crenulata*	[11, 20-22, 25, 27, 30, 31]
Methyl gallate	*R. rosea*	[27]
1,2,3,6-Tetra-*O*-galloyl-*β*-glucose	*R. sachalinensis*	[21]
1,2,3,4,6-Penta-*O*-galloyl-*β*-glucopyranose	*R. henryi, R. sachalinensis, R. crenulata*	[11, 21, 32]
4-Methyl-phenyl-*O*-(6'-*O*-*β*-fructofuranosyl)-*β*-glucopyranoside	*R. sachalinensis*	[33]
Piceoside	*R. pamiera*	[30]
Pyrogallol	*R. crenulata*	[34]
Gallic acid ethyl ester	*R. crenulata*	[35]

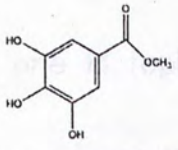
Figure 1-5. Phenolic derivatives isolated from *Rhodiola* species



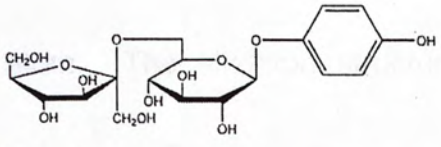
p-Hydroxyacetophenone



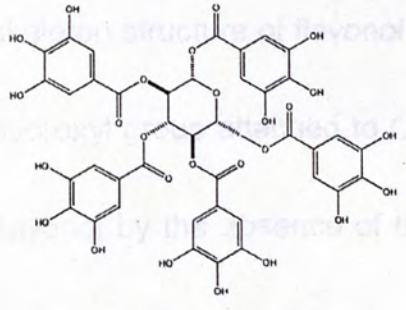
Gallic acid



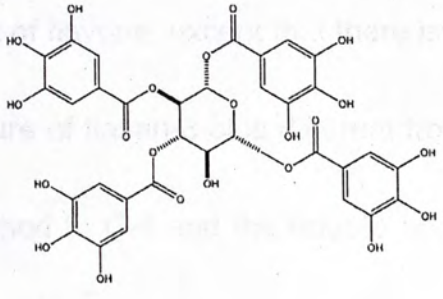
Methyl gallate



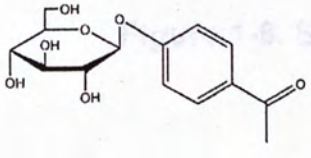
4-Methyl-phenyl-*O*-(6'-*O*-*β*-fructofuranosyl)-*β*-glucopyranoside



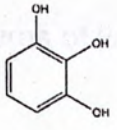
1,2,3,4,6-Penta-*O*-galloyl-*β*-glucopyranose



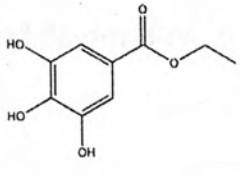
1,2,3,6-Tetra-*O*-galloyl-*β*- glucose



Piceoside



Pyrogallol

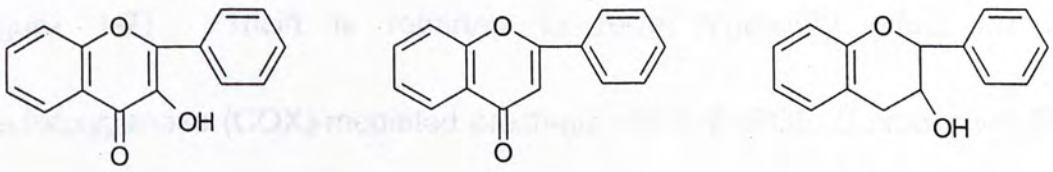


Gallic acid ethyl ester

1.1.4 Flavonoids

Among the compounds isolated from the *Rhodiola* genus, majority are flavonoids. These flavonoids can be categorized into three types, flavonols, flavone and flavan-3-ol. They have skeleton structures consisting of fifteen carbons, forming three-ring structures with two aromatic rings (Figure 1-6). Position one is replaced with an oxygen atom. The skeleton structural differences among these three are on the C ring. In flavones, there is an olefinic linkage between C-2 and C-3, and the C-4 forms a keto group. The skeleton structure of flavonol is similar to that of flavone, except that there is a hydroxyl group attached to C-3. The structure of flavan-3-ol is different from flavonol by the absence of the oxygen attached to C-4 and the double bond between C-2 and C-3.

Figure 1-6. Skeleton structures of flavonol, flavone and flavan-3-ol



Flavonol Flavone Flavan-3-ol

1.1.4.1 Flavone and Flavone Glycosides

Flavones show characteristic NMR spectral patterns. Due to the presence of 2,3-olefinic bond, the chemical shift of C-2 is ranged between δ 160-165, whereas for C-3, the chemical shift is between δ 103-112. The presence of a keto group would cause a chemical shift of δ 176-184 in C-4 [36]. Hydroxyl groups are usually attached to the C-5, C-7, C-3' and C-4'. This would cause the chemical shifts of non-substituted aromatic carbon nearby to fall into the range of δ 110-140. With the presence of hydroxyl group at C-5, chemical shift of C-4 would be between δ 180-182. With the absence of hydroxyl group at C-5, the chemical shift of C-4 would be about δ 175-178 [37]. This is because of the formation of intra-molecular hydrogen bond between the C-4 keto group and the C-5 hydroxyl group. The presence of C-5 hydroxyl group would also upfield shift the resonance of C-3, but to a minor extent [38].

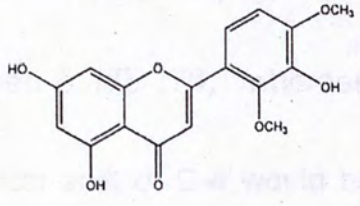
From the literatures, there are three flavones and glycosides found in the eleven species of *Rhodiola*. They are tricin and its glycosides (Table 1-4, Figure 1-7). Tricin is reported to have regulatory effect on the Cyclooxygenase (COX)-mediated prostaglandin E-2 (PGE-2) production [39]. PGE-2 is a prostanoid that participates in cell proliferation, angiogenesis, invasiveness and carcinogenesis. Researchers found that Tricin can inhibit

the enzymatic activity of Cyclooxygenase-1 (COX-1) and Cyclooxygenase -2 (COX-2). This suggests that tricin may act as a potential anti-cancer drug.

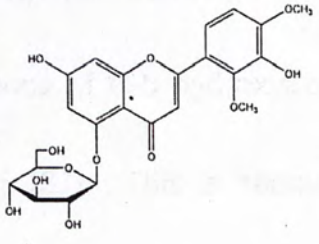
Table 1-4. Flavone and their glycosides from *Rhodiola* species

Name	Plant source(s)	Ref.
Tricin	*R. rosea, R. henryi*	[11, 27]
Tricin-5-glucoside	*R. rosea*	[27]
Tricin-7-glucoside	*R. rosea. R. henryi*	[11, 27]

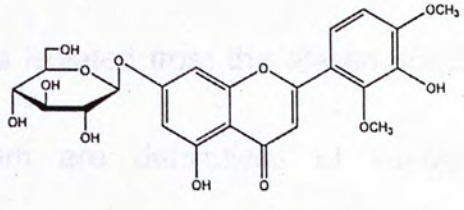
Figure 1-7. Flavone and their derivatives isolated from *Rhodiola* species.



Tricin



Tricin-5-glucoside



Tricin-7-glucoside

1.1.4.2 Flavonols and Their Glycosides

Flavonols are 3-hydroxy derivatives of flavones. Due to the presence of 3-OH, the chemical shifts of C-2 and C-3 behave differently. The characteristic NMR spectral patterns for flavonols are as follows. Because of the presence of oxy-olefinic bond between C-2 and C-3 and the presence of 3-OH, the chemical shift of C-2 is usually observed between δ 145-150, whereas for C-3, the chemical shift is between δ 136-139. The presence of a keto group would cause a chemical shift of δ 172-177 for C-4 [36]. With the presence of hydroxyl group at C-5, the chemical shift of C-4 would be between δ 175-176, whereas with the absence of C-5 hydroxyl group, the chemical shift of C-4 would be about δ 172.5 [37]. This is because of the formation of intra-molecular hydrogen bond between the C-4 keto group and the C-5 hydroxyl group [38].

There are 25 flavonols isolated from the eleven species of *Rhodiola* (Figure 1-8). Many of them are derivatives of kaempferol and herbacetin. Kaempferol, herbacetin and their derivatives are effective free radical scavengers [40]. Kaempferol is also reported to have biphasic effect on estrogenicity [41], which may suggest that kaempferol participates in the homeostasis of estrogen.

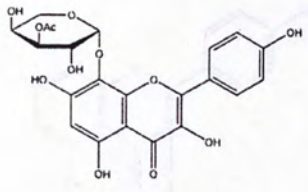
Table 1-5. Flavonols and their glycosides isolated from *Rhodiola* species

Name	Plant source(s)	Ref.
Acetylrhodalgin	*R. rosea, R. algida*	[27, 42]
Crenuloside	*R. dumulosa, R. crenulata*	[35, 43]
Herbacetin-8-methyl ether	*R. atuntsuensis, R. rosea, R. dumulosa*	[20, 27, 44]
Herbacetin-3-*O*-*β*-glucopyranoside	*R. sachalinensis*	[45]
4'-Methoxyl-herbacetin	*R. fastigiata*	[22]
Kaempferol	*R. rosea, R. sachalinensis, R. dumulosa, R. crenulata*	[21, 27, 44, 46]
Kaempferol-3-*O*-*β*-glucopyranoside	*R. sachalinensis*	[45]
Kaempferol-3-*O*-*β*- sophoroside	*R. sachalinensis*	[45]
Kaempferol 3-*O*-*β*-xylofuranosyl(1→2)-*β*-glucopyranoside	*R. sachalinensis*	[21]
Kaempferol 3-*O*-*β*-glucopyranosyl(1→2)-*β*-glucopyranoside	*R. sachalinensis*	[21]
Kaempferol-7-rhamnoside	*R. rosea, R. dumulosa, R. crenulata*	[27, 44, 46]
Rhodalgiside	*R. algida*	[42]
Rhodalgisin	*R. algida*	[42]
Rhodalide	*R. algida*	[42]
Rhodalidin	*R. rosea*	[27]
Rhodalin	*R. rosea*	[27]
Rhodiolatuntoside	*R. atuntsuensis*	[20]
Rhodiolgin	*R. rosea*	[27]

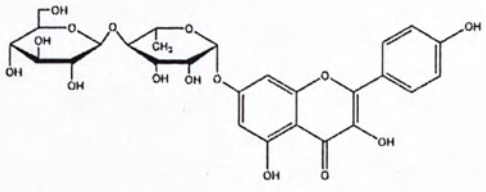
Table 1-5. (Continued)

Rhodiolgidin	*R. rosea*	[27]
Rhodiolin	*R. rosea*	[27]
Rhodionidin	*R. rosea*	[27]
Rhodionin	*R. rosea, R. sachalinensis, R. dumulosa, R. crenulata*	[21, 27, 35, 44]
Rhodiosin	*R. rosea, R. sachalinensis, R. dumulosa, R. crenulata*	[21, 25, 27, 43]
Rutin	*R. atuntsuensis*	[20]
Quercetin	*R. atuntsuensis*	[20]

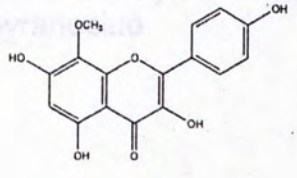
Figure 1-8. Flavonols and their derivatives isolated from *Rhodiola* species



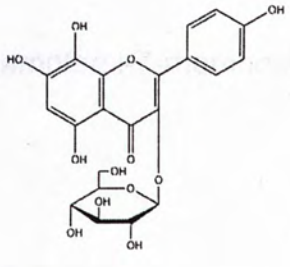
Acetylrhodalgin



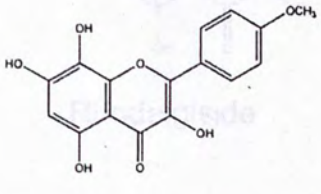
Crenuloside



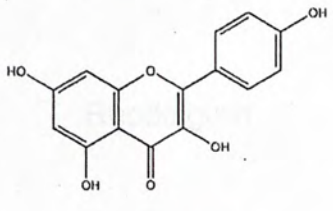
Herbacetin-8-methyl ether



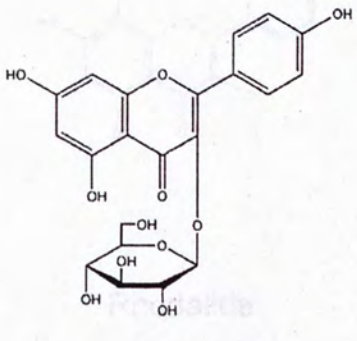
Herbacetin-3-*O*-β-glucopyranoside



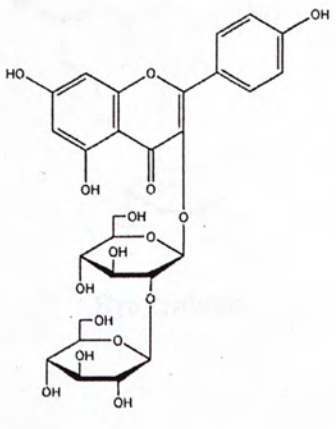
4'-Methoxyl-herbacetin



Kaempferol

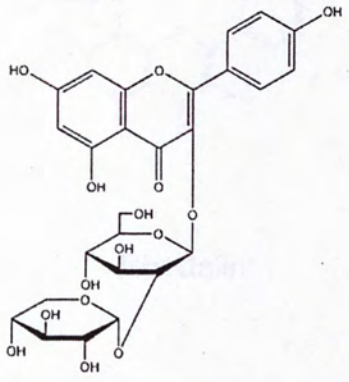


Kaempferol-3-*O*-β-glucopyranoside

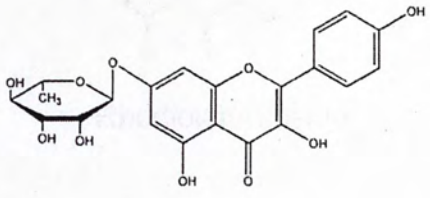


Kaempferol-3-*O*-β-sophoroside

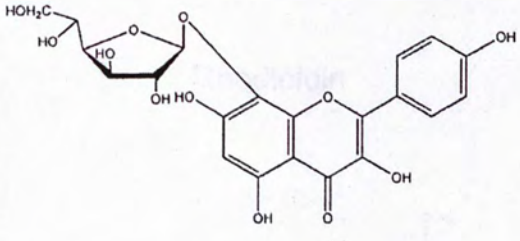
Figure 1-8. (Continued)



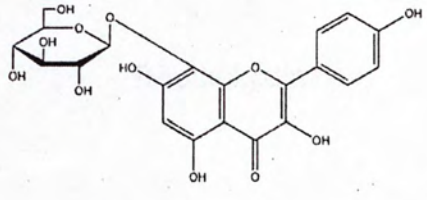
Kaempferol 3-*O*-*β*-xylofuranosyl(1→2) -*β*-glucopyranoside



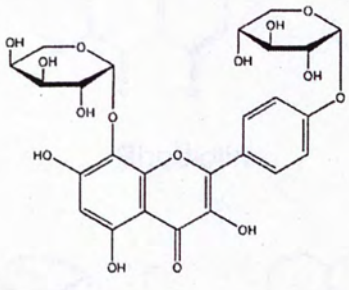
Kaempferol-7-rhamnoside



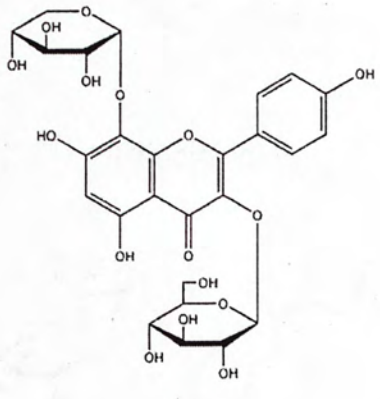
Rhodalgiside



Rhodalgisin

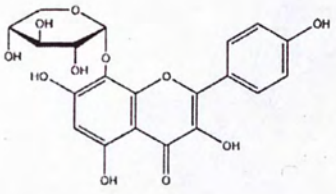


Rhodalide

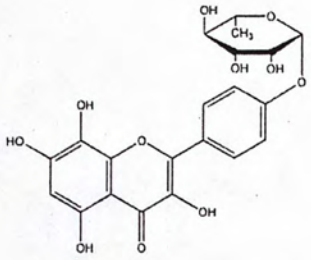


Rhodalidin

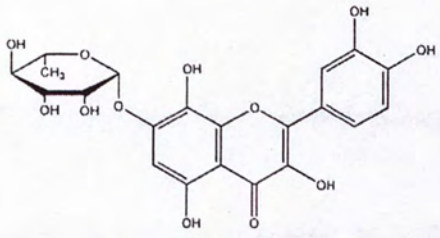
Figure 1-8. (Continued)



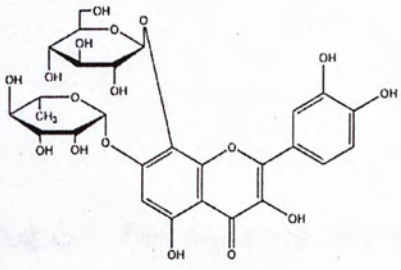
Rhodalin



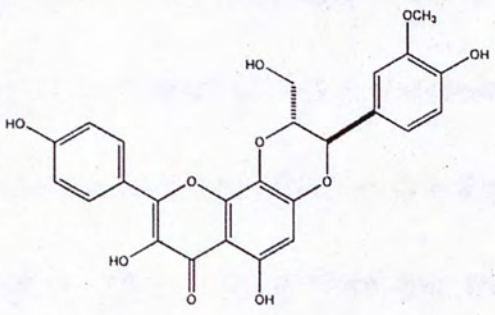
Rhodiolatuntoside



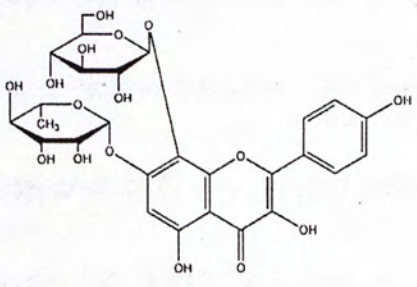
Rhodiolgin



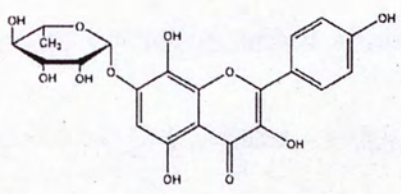
Rhodiolgidin



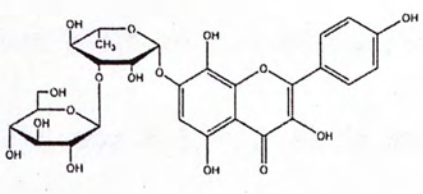
Rhodiolin



Rhodionidin

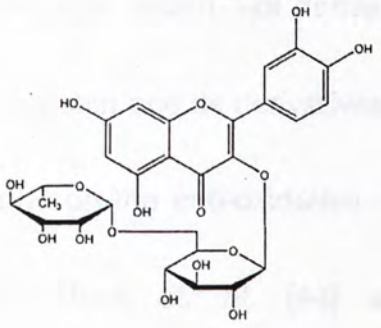


Rhodionin

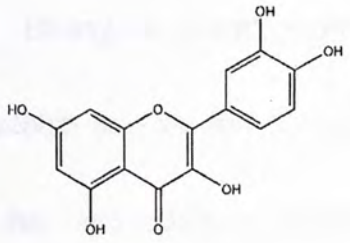


Rhodiosin

Figure 1-8. (Continued)



Rutin



Quercetin

1.1.4.3 Flavan-3-ol Derivatives

Flavan-3-ols are 3-hydroxyl derivatives of flavans. The presence of a single bond between C-2 and C-3 and the absence of keto group at C-4 shape their characteristic NMR spectral patterns. The chemical shift of C-2 falls between δ 76-82, whereas for C-3, the chemical shift is between δ 65-70. Because of the absence of keto group at C-4, the chemical shift of C-4 is ranged between δ 24-32 [38]. Apart from the characteristic ^{13}C NMR spectral pattern, flavan-3-ols also show characteristic ^{1}H NMR spectral pattern of the C-ring. The two H-4 protons would show double-doublet signals. In addition, being influenced by the spatial configuration of H-2 and H-3, H-2 would appear either as a singlet (*cis*) or doublet (*trans*) on the NMR spectrum. H-3 is influenced by H-2 and H-4 protons, and appears as a multiplet signal on the

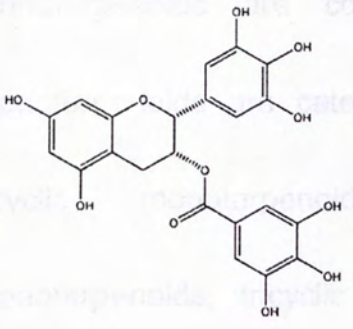
^{1}H NMR spectrum [47].

There are four flavan-3-ol derivatives reported from *Rhodiola* plants. They are epicatechin and its derivatives (Table 1-6). Biological investigations have been done on the anti-oxidative effect of epicatehin and its derivatives. For example, Rizvi *et. al.* [48] investigated the anti-oxidative activity of epigallocatechin gallate (EGCG), epigallocatechin (EGC), epicatechin gallate (ECG) and epicatechin (EC) on erythrocytes under oxidative stress induced by tert-butylhydroperoxide (t-BHP). They found out the relative effectiveness of anti-oxidative potential of each epicatehin are in the order of EGCG > ECG > EGC > EC.

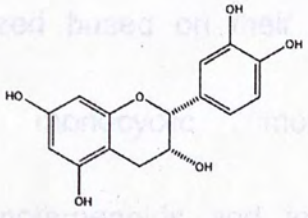
Table 1-6. Flavan-3-ol and their glycosides isolated from *Rhodiola* species

Name	Plant source	Ref.
Epicatechin	*R. yunnanensis*	[21, 31]
(-)-Epigallocatechin	*R. sachalinensis*	[21]
Epigallocatechin 3-gallate	*R. yunnanensis*	[31]
3-*O*-Galloylepigallocatechin-(4→8) -epigallocatechin 3-*O*-gallate	*R. sachalinensis*	[21]

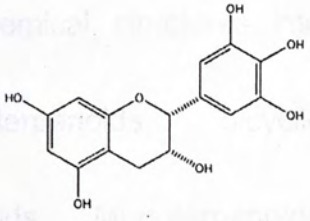
Figure 1-9. Flavan-3-ol and their derivatives isolated from *Rhodiola* species



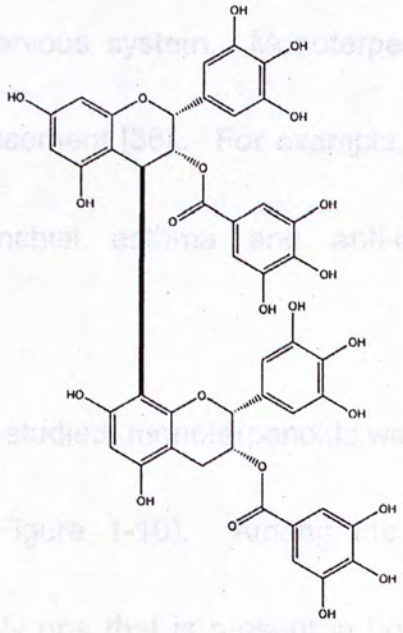
Epigallocatechin 3-gallate



Epicatechin



(-)-Epigallocatechin



3-*O*-Galloylepigallocatechin-(4→8)-epigallocatechin 3-*O*-gallate

1.1.5 Monoterpenoids

Monoterpenoids are compounds possessing a ten-carbon skeleton. Monoterpenoids are categorized based on their chemical structures into acyclic monoterpenoids, monocyclic monoterpenoids, bicyclic monoterpenoids, tricyclic monoterpenoids and iridoids. Monoterpenoids have diversified biological effects. They have been found to elicit biological effects on various systems such as the circulatory system, respiratory system, digestive system and nervous system. Monoterpenoids also participate in immune response enhancement [36]. For example, piperitone and camphor can be used in bronchial asthma and anti-inflammation treatments respectively.

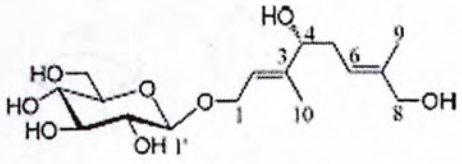
From the eleven species studied, monoterpenoids were found only in *R. rosea* and *R. sachalinensis* (Figure 1-10). Among the twelve monoterpenoids found, rosiridin is the only one that is present in both species. Most of the monoterpenoids found in the genus are acyclic monoterpenoids, except sachalinols B, sachalinols C and achalinosides B, which are monocyclic monoterpenoids.

Table 1-7. Monoterpenoids isolated from *Rhodiola* species

Name	Plant source(s)	Ref.
(2*E*,6*E*,4*R*)-4,8-Dihydroxy-3,7-dimethyl-2,6-octadienyl *β*-glucopyranoside	*R. rosea*	[49]
(2*E*,4*R*)-4-Hydroxy-3,7-dimethyl-2,6-octadienyl *α*- glucopyranosyl(1→6)-*β*-glucopyranoside	*R. rosea*	[49]
(2*E*,4*R*)-4-Hydroxy-3,7-dimethyl-2,6-octadienyl *β*- glucopyranosyl(1→3)-*β*- glucopyranoside	*R. rosea*	[49]
(2*E*,4*R*)-4,7-Dihydroxy-3,7-dimethyl-2-octenyl *β*- glucopyranoside	*R. rosea*	[49]
(2*E*)-7-Hydroxy-3,7-dimethyl-2-octenyl *α*-arabinopyranosyl(1→6)-*β*- glucopyranoside	*R. rosea*	[49]
Rosiridol	*R. rosea*	[27]
Rosiridin	*R. rosea*, *R. sachalinensis*	[21, 27]
Sachalinol A	*R. sachalinensis*	[21]
Sachalinol B	*R. sachalinensis*	[21]
Sachalinol C	*R. sachalinensis*	[21]
Sachalinoside A	*R. sachalinensis*	[21]
Sachalinoside B	*R. sachalinensis*	[21]

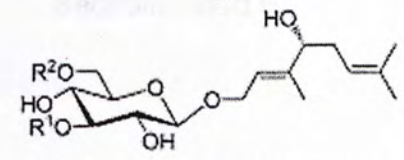
Figure 1-10. Monoterpenoids isolated from *Rhodiola* species

1: (2*E*, 6*E*, 4*R*)-4,8-dihydroxy-3,7-dimethyl-2,6-octadienyl *β*-glucopyranoside



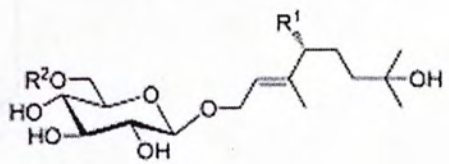
1

2: (2*E*, 4*R*)-4-hydroxy-3,7-dimethyl-2,6-octadienyl *α*- glucopyranosyl(1→6)-*β*-glucopyranoside.
3:(2*E*, 4*R*)-4-hydroxy-3,7-dimethyl-2,6-octadienyl *β*- glucopyranosyl(1→3)-*β*-glucopyranoside

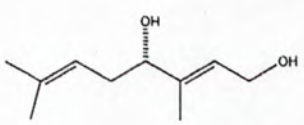


2 R^1 = H R^2 = α-D-glucopyranosyl
3 R^1 = β-D-glucopyranosyl R^2 = H

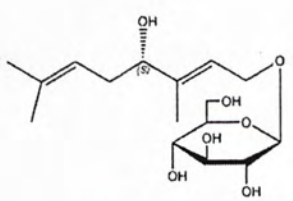
4: (2*E*, 4*R*)-4,7-dihydroxy-3,7-dimethyl-2-octenyl *β*- glucopyranoside
5: (2*E*)-7-hydroxy-3,7-dimethyl-2-octenyl *α*-L-arabinopyranosyl(1→6)-*β*-glucopyranoside



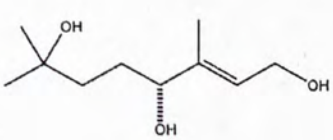
4 R^1 = OH R^2 = H
5 R^1 = H R^2 = α-L-arabinopyranosyl



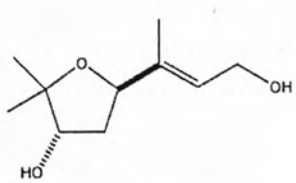
Rosiridol



Rosiridin

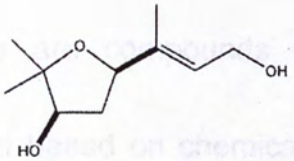


Sachalinol A

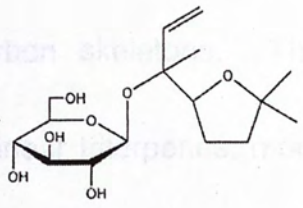


Sachalinol B

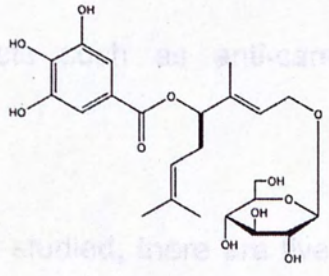
Figure 1-10. (Continued)



Sachalinol C



Sachalinoside B



Sachalinoside A

1.1.6 Triterpenes

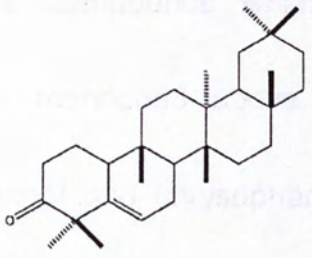
Triterpenes are compounds containing 30-carbon skeletons. They are categorized based on chemical structures into linear triterpenes, monocyclic triterpenes, bicyclic triterpenes, tricyclic triterpenes, tetracyclic triterpenes and pentacyclic triterpenes. Similar to monoterpenoids, triterpenes have very diversified biological effects such as anti-cancer, anti-inflammation and anti-virus properties [36].

Among the eleven species studied, there are five triterpenes recorded in the literature (Figure 1-11). All five triterpenes belong to pentacyclic triterpenes. Glutin-5-en-3-one, taraxeryl acetate and isomultiforenyl acetate belong to oleanane type. Isomotiol and isomotiol acetate belong to gammacerane type.

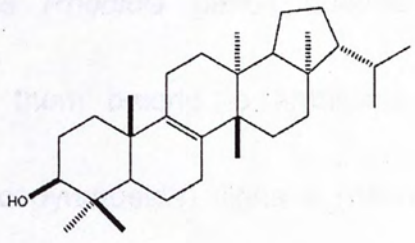
Table 1-8. Monoterpenoids from *Rhodiola* genus

Name	Plant source(s)	Ref.
Glut-5-en-3-one	*R. pamiera*	[30]
Isomotiol	*R. sachalinensis*	[21]
Isomotiol acetate	*R. henryi*	[50]
Isomultiforenyl acetate	*R. henryi*	[50]
Taraxeryl acetate	*R. sachalinensis, R. henryi*	[28, 50]

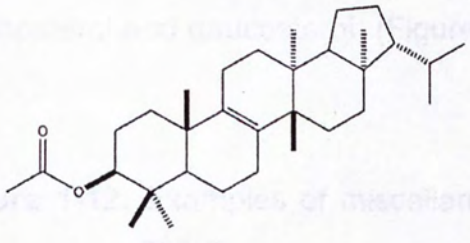
Figure 1-11. Triterpenes isolated from *Rhodiola* species



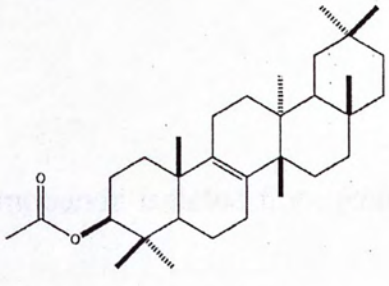
Glut-5-en-3-one



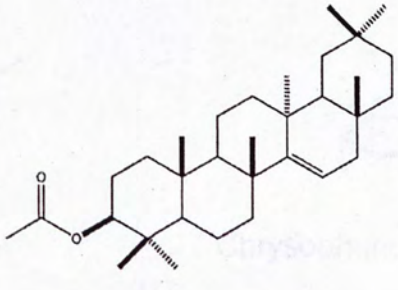
Isomotiol



Isomotiol acetate



Isomultiforenyl acetate

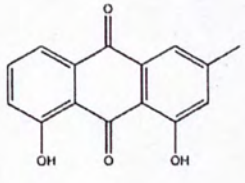


Taraxeryl acetate

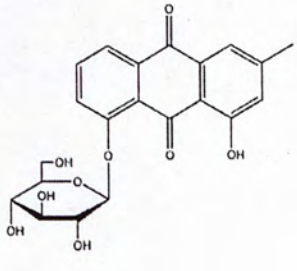
1.1.7 Miscellaneous Compounds

There are compounds isolated from the *Rhodiola* genus outside the categories mentioned above. Some of them belong to anthraquinone (chrysophanol and chrysophanol-8-*O*-*β*-glucopyranoside), lignans (cedrusin, (+)-isolariciresinol and (+)-isolariciresinol-9-*O*-*β*-glucopyranoside) and steroids (*β*-sitosterol and daucosterol) (Figure 1-12).

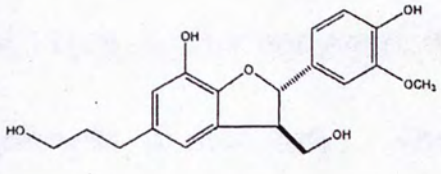
Figure 1-12. Examples of miscellaneous compounds isolated from *Rhodiola* genus



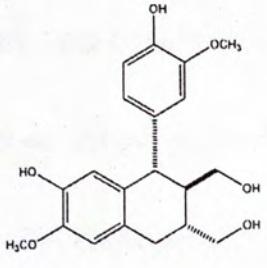
Chrysophanol



Chrysophanol-8-*O*-*β*-glucopyranoside

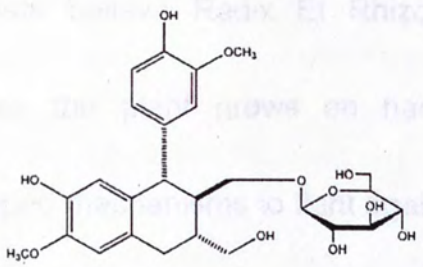


Cedrusin

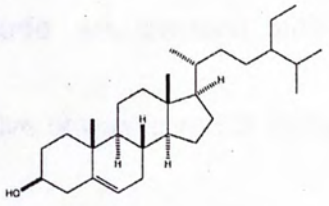


(+)-Isolariciresinol

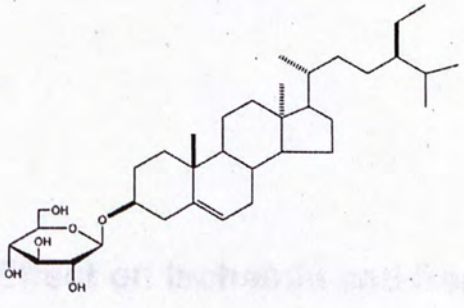
Figure 1-12. (Continued)



(+)-Isolariciresinol-9-*O*-*β*-glucopyranoside



β-Sitosterol



Daucosterol

1.2 Biological Activities of *Rhodiola* Genus

Salidroside, tyrosol, rhodionin, rosavin and rosarin are considered the active ingredients of the herb. They play significant roles in anti-oxidation, anti-cancer, enhancement of immune response and improvement of learning and memory.

1.2.1 Anti-oxidative Effect

Scientists believe Radix Et Rhizoma Rhodiolae has anti-oxidizing effect because the plant grows on harsh high altitude environment and has developed mechanisms to fight against the oxidative stress from UV radiation. Researches have shown that it can protect the heart from oxidative stress after ischemia and reperfusion [51]. In addition, the herb is found to have anti-aging effect [52].

1.2.1.1 Protective Effect on Ischemia and Reperfusion

Zou [51] investigated the effect of salidroside on the primary culture of rat cardiomyocytes upon ischemia / reperfusion stimulated oxidative injury. They investigated the change in medium lactate dehydrogenase (LDH) and malondialdehyde (MDA) concentration. Under oxidative injury, cells may die and leak intracellular enzyme LDH to the surrounding medium. MDA, on the other hand, is an end product of lipid peroxidation, which is induced by reactive oxygen species (ROS). Lipid peroxidation acts like ROS, which exerts further oxidative stress on cells. Salidroside can effectively reduce cell deaths and the generation of MDA under oxidative stress.

Song *et. al.* [53] used an induced myocardial ischemia / reperfusion injury model to study the protective effect of *Rhodiola* sa*chalinensis* extract. In the *Rhodiola* treatment group, the concentration of nitric oxide (NO, an ROS that the concentration will increase upon ischemia / reperfusion injury) and MDA was significantly lower than the untreated group, and the superoxide dismutase (SOD) level was higher after treatment. This showed the protective effect of *Rhodiola* on myocardial ischemia / reperfusion injury by lowering the generation of ROS and increasing the level of anti-oxidizing enzymes.

1.2.1.2 Anti-Aging Effect

Superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase are typical anti-oxidizing enzymes. Lipofuscin is a substance synthesized in liver and accumulated underneath the skin in aged human, giving to brown black spots. The herb extract is found to increase the SOD, GSH-Px and catalase activities to decrease the accumulation of lipofuscin and MDA in aged mice [52, 54].

Besides, Arora *et. al.* [55] investigated the radio-protective effect of *Rhodiola imbricata*. They found that the rhizome extract of *Rhodiola imbricata* had

superoxide ion scavenging, metal chelation, anti-oxidizing and anti-lipid peroxidation effects. Moreover, the extract can protect erythrocytes from hemolysis under radiation by preventing radiation-induced member degeneration.

1.2.2 Learning and Memory

Learning and memory is an extremely complex process. Many neuropeptidases in the brain are involved. Prolyl endopeptidase (PEP) is an enzyme that participates in the catabolism of a proline-containing neuropeptidase that may play a role in learning and memory. Therefore, the inhibition of the PEP helps improve learning and memory. Fan *et. al.*[56] tested over twenty compounds from *R. sachalinensis*, and found out that 1,2,3,6-tetra-O-galloyl-β-D-glucose, 1,2,3,4,6-penta-O-galloyl-β-D-glucose, rhodionin, rhodiosin, 3-O-galloylepigallocatechin-(4 $\rightarrow$ 8)-epigallocatechin 3-O-gallate and rosiridin have inhibitory effect on PEP.

Animal model has also been set up to investigate the effect of this drug on learning and memory. Liu *et. al.* [57] treated mice groups with *Rhodiola henryi* extract for thirty days. In step-down test, dark avoidance test and water-maze test, *Rhodiola* treated group showed a lower mistake rate when compared to the untreated group.

1.2.3. Immune Response

When human body is invaded by pathogens, inflammation will be triggered. During inflammation, the permeability of endothelial layer increases, leading to the outflux and accumulation of tissue fluid at the infected area, which will cause flush and pain. Meanwhile, white blood cells such as macrophages and natural killer cells (NK cells) will travel to the infected area and kill the pathogens. However, many cytokines and other cytotoxic substances will be released at the same time, causing necrosis. Radix Et Rhizoma Rhodiolae was found to be able to decrease the permeability of the endothelial layer, and reduce the edema symptom. It also increases the antibody production and the endocytosis activity of macrophages and NK cells, which helps to destroy the pathogens more effectively [58].

Another study used *Rhodiola rosea* extract to investigate the effect of drug extract on immune system. Zhao *et. al.* [59] found that *Rhodiola* extract can increase the concentration of T lymphocytes, macrophages and plaque-forming cells in mice, which suggested an enhancement of the immune system.

1.2.4 Anti-cancer Effect

Cancer is a lethal disease because of the uncontrolled proliferation and the rapid colonization of cancer cells into different parts of the body, which harm the normal cells nearby. Therefore, potential anti-cancer drugs are formulated to inhibit the proliferation and colonization of cancer cells, as well as to exert lethal effect to cancer cells. Luo *et. al.* [60] tested the *Rhodiola* extract on hepatic carcinoma cell line QGY-7703. They found out that *Rhodiola* extract can inhibit the proliferation and colonization of cancer cells. Their experiment revealed that *Rhodiola* extract can alter the membrane potential of cancer cells, which causes cancer cells to lose the adhesion ability and fail to colonize in different parts of the body. They also reported that *Rhodiola* extract can inhibit the uptake of TdR into the cancer cells, which is one of the nucleotides required for DNA synthesis during cell proliferation.

1.3 Objective

This project aims to isolate and determine the structure of pure compounds from one of the medicinal species of Radix Et Rhizoma Rhodiolae, *Rhodiola kirilowii*.

Chapter 2 Experimental

2.1 General Experimental Procedures

TLC was carried out on silica gel-60 F_{254} and RP-18 F_{254} plates. Column chromatography was carried out on silica gel, RP-18 silica gel, Sephadex LH-20, D-101 macroporous resin and Diaion HP-20 resin. HPLC was done on Hewlett Packard 1100 series HPLC. Compound purity was analyzed by HPLC and TLC using at least two solvent systems. Each pure compound displayed as a single peak on HPLC and as a single spot on TLC. ^{1}H and ^{13}C NMR data were obtained from a Bruker Avance NMR spectrometer at 400 and 100 MHz respectively. Molecular mass data was obtained from Agilent 1100 series HPLC coupled with an Agilent 1100 series LC/MD trap mass spectrometer via an APCI interface.

2.2 Plant Materials

The dry roots of *Rhodiola kirilowii* (Regel) Maxim. were collected in Gan Su province at altitude of about 2500 m, and identified by Ms. Zong Yu Ying of the School of Chinese Medicine, The Chinese University of Hong Kong.

2.3 Extraction and Isolation

Dry roots of *R. kirilowii* (10 kg) were ground into powder and macerated by 80% aqueous ethanol (30 litres) for one week. The solution was filtered and evaporated to dryness using a rotary evaporator under reduced pressure. The residue was re-dissolved in water and partitioned with 2.5 litres of hexane for three times, 2.5 litres of ethyl acetate for five time, and 2.5 litres of butanol for five times successively, to obtain hexane-soluble (6.5 g), ethyl acetate-soluble (120 g), butanol-soluble (250 g) and water-soluble fractions.

2.3.1 Isolation and Purification of the Ethyl Acetate (E.A.) Fraction

The E.A. fraction was subjected to silica gel column using a dichloromethane : methanol gradient with increasing polarity (dichloromethane : methanol 100:0 → 0:100). Eight fractions were eluted (fraction 1 to 8).

Fraction 1 was subjected to silica gel column and eluted with hexane : ethyl acetate gradient with increasing polarity (hexane : ethyl acetate 100:0, 98:2, 97:3, 95:5, 92:8 90:10, 80:20, and 100% methanol). Compound **1** was eluted from hexane : ethyl acetate (98:2).

Fraction 3 was subjected to silica gel column and eluted with hexane : acetone with increasing polarity (hexane : acetone 100:0, 95:5, 93:7, 90:10, 85:15, 80:20 and 100% methanol). Compound **2** was in a fraction eluted from hexane : acetone (90:10).

Fraction 4 was subjected to sephadex LH-20 column and eluted with 100% methanol. Fractions 4-1 to 4-7 were collected. Fraction 4-7 was further purified with RP-18 column and eluted with methanol : water gradient with decreasing polarity (methanol : water 5:95, 8:92, 10:90, 100:0). Fraction 4-7-1 to 4-7-7 were collected. Fraction 4-7-4 was then subjected to RP-18 column and eluted with methanol : water gradient with decreasing polarity (methanol : water 0:100, 5:95, 20:80, 25:75, 30:70, 45:55 and 50:50). Compound **3** was purified from methanol : water (30:70).

Fraction 5 was subjected to Diaion HP-20 resin column and eluted with methanol : water gradient with decreasing polarity (methanol : water 0:100, 10:90, 30:70, 50:50, 60:40, 70:30, 80:20 and 100:0). Fractions 5-1 to 5-7 were collected. Fraction 5-3 was then purified by RP-18 HPLC by eluting with methanol : water (30 : 70) to obtain compound **4** and **5**. On the other hand, fraction 5-4 was further separated on a RP-18 column and eluted with methanol : water gradient with decreasing polarity (methanol : water 10:90,

20:80, 30:70, 40:60, 42:58, 45:55, 50:50 55:45 and 100:0) Compound **6** was purified from methanol : water (50:50).

Fraction 6 was subjected to silica gel column and eluted with dichloromethane : methanol gradient with increasing polarity (dichloromethane : methanol 100:0, 99:1, 97:3, 95:5, 93:7 90:10 and 0:100) Fractions 6-1 to 7 were collected. Fraction 6-3 was then subjected to sephadex LH-20 column and eluted with 100% methanol to afford compound **7**.

Fraction 8 was subjected to silica gel column and eluted with ethyl acetate : methanol gradient with increasing polarity (acetate : methanol 100:0, 95:5, 90:10 and 0:100). Fraction 8-1 to 8-5 were collected. Fraction 8-1 was further separated on sephadex LH-20 to yield compound **8**. Fraction 8-2 was passed through a sephadex LH-20 column eluted with methanol. Three fractions, 8-2-1 to 8-2-3, were collected. Fraction 8-2-2 was further purified on a RP-18 column and eluted with methanol : water gradient with decreasing polarity (methanol : water 30:70, 50:50 and 100:0). Three fractions were collected as fraction 8-2-2-1 to 8-2-2-3. Fraction 8-2-2-2 was then subjected to sephadex column and eluted with 100% methanol. Two fractions (8-2-2-2-1 and 8-2-2-2-2) were collected. Finally, fraction 8-2-2-2-2 was purified by

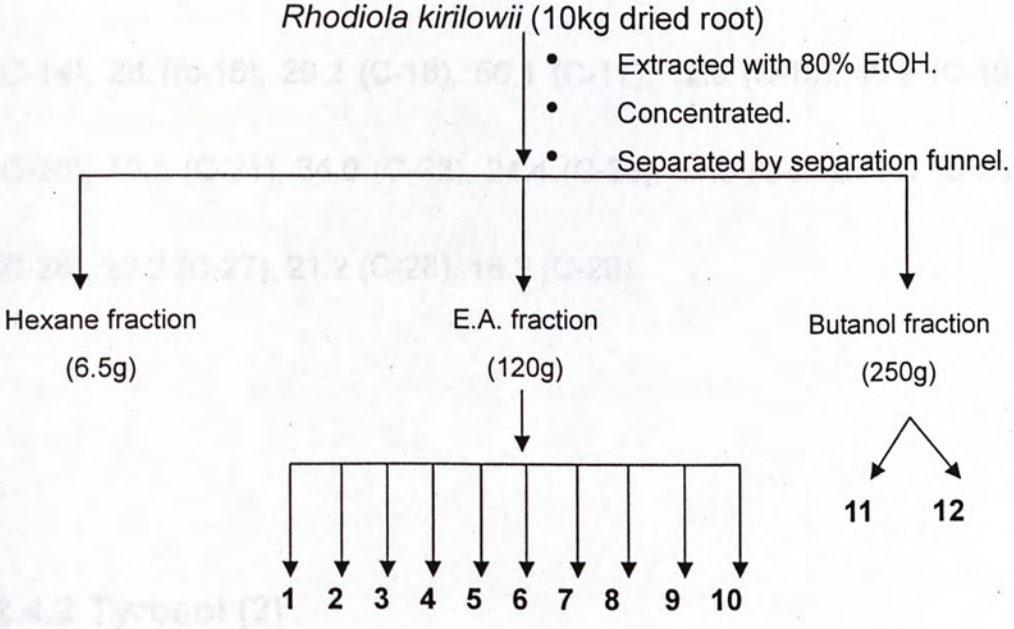
RP-18 chromatography and eluted with methanol : water gradient (methanol : water 30:70, 40:60, 42:58 and 100:0). Compound **9** was eluted from methanol : water (42:58). On the other hand, fraction 8-3 was subjected to RP-18 column and eluted with methanol : water gradient with decreasing polarity (methanol : water 10:90, 15:85, 20:80, 25:75, 30:70 and 100:0). Six fractions were collected (8-3-1 to 8-3-6). Fraction 8-3-5 was then passed through a to RP-18 column and eluted with methanol : water gradient with decreasing polarity (methanol : water 10:90 18:92, 24:76, 28:72, 35:65, 45:55, 50:50 and 100:0). Compound **10** was isolated from the fraction of at methanol : water (20:80).

2.3.2 Isolation and Purification of the Butanol Fraction

The butanol fraction was subjected to Diaion HP-20 resin column and eluted with methanol : water gradient with decreasing polarity (methanol : water 0:100, 15:85, 30:70, 45:55, 60:40, 80:20 and 100:0). Four fractions were collected (Fraction 1 to 4).

Fraction 4 was separated on D-101 macroporous resin and eluted with methanol : water gradient with decreasing polarity (methanol : water 0:100, 14:85, 30:70, 40:60, 50:50, 60:40, 75:25, 100:0). Fraction 4-1 to 4-3 were

collected. Fraction 4-2 was further purified by RP-18 column and eluted with methanol : water gradient with decreasing polarity (methanol : water 10:90, 20:80, 30:70, 40:60, 50:50, 60:40 and 100:0). Fraction 4-2-1 to 4-2-3 were collected. Fraction 4-2-2 was then subjected to RP-18 HPLC and eluted with methanol : water (32:68) to obtain compound **11** and **12**.



2.4 Characterization of the Isolated Compounds

2.4.1 β-Sitosterol (1)

Colorless needles, $C_{29}H_{50}O$, UV λ_{max}: 266 nm; ^1H NMR: ($CDCl_3$, 400MHz) δ 5.32 (1H, *m*, H-6), 3.5 (1H, *m*, H-3α); ^{13}C NMR ($CDCl_3$, 100MHz) δ 36.6(C-1), 29.2 (C-2), 71.8 (C-3), 45.9 (C-4), 140.6 (C-5), 121.6(C-6), 31.7 (C-7), 32.0 (C-8), 50.2 (C-9), 36.2 (C-10), 28.3 (C-11), 39.8 (C-12), 42.4 (C-13), 56.8 (C-14), 26.1(c-15), 29.2 (C-16), 56.1 (C-17), 12.0 (C-18), 19.9 (C-19), 32.0 (C-20), 19.5 (C-21), 34.0 (C-22), 24.4 (C-23), 37.3 (C-24), 19.1 (C-25), 12.1 (C-26), 23.2 (C-27), 21.2 (C-28), 18.9 (C-29).

2.4.2 Tyrosol (2)

Colorless needles, $C_8H_{10}O_2$, UV λ_{max}: 274 nm, 232nm; ^1H NMR (CD_3OD, 400MHz) δ 7.01(2H, *d* , *J* = 8.8 Hz, H-4, 8), 6.69 (2H, *d*, *J* = 8.3 Hz, H-5, 7), 3.67 (2H, *t*, *J* = 7.1 Hz, H-1), 2.70 (2H, *t*, *J* = 7.1 Hz, H-2); ^{13}C NMR (CD_3OD, 100MHz) δ 64.5 (C-1), 39.4 (C-2), 130.7 (C-3, 4, 8), 116.0 (C-5, 7), 156.5 (C-6).

2.4.3 *trans*-Hydroxycinnamic acid (3)

White needles, $C_9H_8O_3$, APCI-MS *m/z*: 165 $[M+H]^+$; UV λ_{max}: 226 nm, 310 nm; ^1H NMR (CD_3OD, 400 MHz) δ 6.27 (1H, *d*, *J* = 15.6 Hz, H-2), 7.59 (1H, *d*, *J* = 15.6 Hz, H-3), 7.43 (2H, *d*, *J* = 8.4 Hz, H-5, 9), 6.89 (2H, *d*, *J* = 8.4 Hz, H-6, 8); ^{13}C NMR (CD_3OD, 100MHz) δ 171.5 (COOH) , 116.1 (C-2), 147.1(C-3), 127.7(C-4), 131.6(C-5, 9), 117.3(C-6, 8), 161.6(C-7).

2.4.4 Geranyl-*β*-glucopyranoside (4)

Brownish-yellow powder, $C_{16}H_{28}O_6$, UV λ_{max}: 210 nm; ^1H NMR (CD_3OD, 400 MHz) δ 4.18 - 4.34 (2H, *m*, H-1a, H-1b), 5.37 (1H, *m*, H-2), 2.0 – 2.2 (4H, *m*, H-4, H-5), 5.10 (1H, *m*, H-6), 1.60 (3H, *s*, H-8), 1.67 (3H, *s*, H-9), 1.75 (3H, *s*, H-10), 4.28 (1H, *d*, *J* = 8 Hz, Glc-1H), 3.13 – 3.91 (6H, Glc-2H to Glc-6H); ^{13}C NMR (CD_3OD, 100MHz) δ 66.8 (C-1), 122.1 (C-2), 142.3 (C-3), 41.2 (C-4), 27.9 (C-5), 125.6 (C-6), 133.0 (C-7), 18.3 (C-8), 26.4 (C-9), 17.0 (C-10), 103.2 (Glc-1), 75.6 (Glc-2), 78.7 (Glc-3), 72.2 (Glc-4), 78.5 (Glc-5), 63.3 (Glc-6).

2.4.5 Neryl-β-glucopyranoside (5)

Brownish-yellow powder, $C_{16}H_{28}O_6$, UV λ_{max}: 210 nm; ^{1}H NMR (CD_3OD, 400 MHz) δ 4.22 - 4.36 (2H, *m*, H-1a, H-1b), 5.36 (1H, *t*, *J* = 3.2, 3.2 Hz, H-2), 2.0 – 2.2 (4H, *m*, H-4, H-5), 5.11 (1H, *t*, *J* = 2.8, 2.8 Hz, H-6), 1.60 (3H, *s*, H-8), 1.67 (3H, *s*, H-9), 1.68 (3H, *s*, H-10), 4.28 (1H, *d*, *J* = 8 Hz, Glc-1H), 3.14 - 3.88 (6H, Glc-2H to Glc-6H); ^{13}C NMR (CD_3OD, 100MHz) δ 66.7 (C-1), 123.0 (C-2), 142.3 (C-3), 33.6 (C-4), 28.2 (C-5), 125.5 (C-6), 133.3 (C-7), 18.3 (C-8), 26.4 (C-9), 24.2 (C-10), 103.4 (Glc-1), 75.5 (Glc-2), 78.6 (Glc-3), 72.1 (Glc-4), 78.4 (Glc-5), 63.2 (Glc-6).

2.4.6 Hexyl β-Glucopyranoside (6)

Pale yellow needles, $C_{12}H_{24}O_6$; ^{1}H NMR (CD_3OD, 400MHz) δ 3.17 (2H, *d*, *J* = 7.2 Hz, H-1), 1.21 to 1.60 (8H, H-2 to H-5), 0.80 (3H, *t*, *J* = 6.6 Hz, H-6), 4.15 (1H, *d*, *J* = 7.6 Hz, Glc-1H); ^{13}C NMR (CD_3OD, 100MHz) δ 69.5 (C-1), 29.4 (C-2), 25.7 (C-3), 31.6 (C-4), 22.3 (C-5), 13.0 (C-6), 103.0 (Glc-1), 73.7 (Glc-2), 76.7 (Glc-3), 70.2 (Glc-4), 76.5 (Glc-5), 61.4 (Glc-6).

2.4.7 Gallic Acid (7)

Colourless needles, $C_7H_6O_5$, APCI-MS *m/z*: 169 $[M-H]^-$; UV λ_{max}: 236 nm, 270nm, 304nm; ^1H NMR (CD_3OD, 400MHz) δ 7.07 (2H, *s*, H-2, 6); ^{13}C NMR (CD_3OD, 100MHz) δ 121.8 (C-1), 110.2 (C-2, 6), 146.2 (C-3, 5), 139.3 (C-4), 170.1 (C-7).

2.4.8 Epigallocatechin-3-Gallate (8)

Brown powder, $C_{22}H_{18}O_{11}$, APCI-MS *m/z*: 459 $[M+H]^+$; UV λ_{max}: 208 nm, 210nm, 274nm; $[\alpha]^{20}_D$ -306.7° (*c* 0.75, $CHCl_3$: CH_3OH = 1:1); ^1H NMR (CD_3OD, 400MHz) δ 4.96 (1H, *s*, H-2), 5.52 (1H, *m*, H-3), 2.97(1H, *dd*, *J* = 17.6, 4.4 Hz, H-4a), 2.83 (1H, *dd*, *J* = 17.8, 1.2 Hz, H-4b), 5.95 (1H, *d*, *J* = 0.8 Hz, H-6), 5.95 (1H, *d*, *J* = 0.8 Hz, H-8), 6.49 (1H, *s*, H-2'), 6.49 (1H, *s*, H-6'), 6.94 (2H, *s*, galloyl-H2, 6); ^{13}C NMR (CD_3OD, 100MHz) δ 78.6 (C-2), 69.8 (C-3), 26.9 (C-4), 157.6 (C-5), 96.4 (C-6), 157.6 (C-7), 95.8 (C-8), 157.0 (C-9), 99.3 (C-10), 130.8 (C-1'), 106.7 (C-2'), 146.1 (C-3'), 133.6 (C-4'), 146.1 (C-5'), 106.7 (C-6'), 121.3 (G-1), 110.1 (G-2), 146.5 (G-3), 140.0 (G-4), 146.5 (G-5), 110.1 (G-6), 167.4 (COO).

2.4.9 Rhodiolgin (9)

White crystal, $C_{21}H_{20}O_{12}$, APCI-MS *m/z*: 465 $[M+H]^+$; UV λ_{max}: 206 nm, 260nm, 386nm; ^1H NMR (CD_3OD, 400MHz) ; δ 6.65 (1H, *s*, H-6), 7.85 (1H, *s*, H-2'), 6.89 (1H, *d*, *J* = 4.4 Hz, H-5'), 7.77 (1H, *d*, *J* = 4.4 Hz, H-6'), 5.52 (1H, *s*, rha-1), 3.5 – 4.1 (4H, *m*, rha-2 – rha-5), 1.26 (3H, *d*, *J* = 3 Hz, rha-6); ^{13}C NMR (CD_3OD, 100MHz) δ 148.5 (C-2), 137.2 (C-3), 177.5 (C-4), 151.3 (C-5), 99.0 (C-6), 153.6 (C-7), 128.3 (C-8), 146.0 (C-9), 105.9 (C-10), 124.1 (C-1'), 116.0 (C-2'), 146.0 (C-3'), 148.8 (C-4'), 116.0 (C-5'), 122.1 (C-6'), 100.8 (rha-1), 71.7 (rha-2), 72.0 (rha-3), 73.7 (rha-4), 71.1 (rha-5), 18.1 (rha-6).

2.4.10 Isolariciresinol-9-*β*-Glucopyranoside (10)

White crystal, $C_{26}H_{34}O_{11}$, APCI-MS *m/z*: 540 $[M+H_2O]^+$; UV λ_{max}: 206 nm, 284nm; ^1H NMR (CD_3OD, 400MHz) δ 6.69 (1H, *s*, H-2), 6.65 (1H, *d*, *J* = 4.2 Hz, H-5), 6.53 (1H, *d*, *J* = 0.8 Hz, H-6), 3.96 (1H, *d*, *J* = 3.8 Hz, H-7), 1.76 (1H, *m*, H-8), 3.39 (1H, *m*, H-9a), 3.60 (1H,*d*, *J* = 3 Hz, H-9b), 6.56 (1H, *s*, H-2'), 6.08 (1H, s, H-5'), 2.74 (1H, *d*, *J* = 3.6Hz, H-7a'), 2.73 (1H, *m*, H-7b'), 1.76 (1H, m, H-8'), 3.35 (1H, m, H-9'a), 3.63 (1H, *d*, *J* = 3 Hz, H-9'b), 3.72 (3H, *s*, 3-OCH_3), 3.71 (3H, *s*, 3'-OCH_3), 3.96 (1H, *d*, *J*= 7.6 Hz, Glc-H1); ^{13}C NMR (CD_3OD, 100MHz), δ 137.2 (C-1), 112.9 (C-2), 147.5 (C-3), 144.5 (C-4), 114.7 (C-5), 121.7 (C-6), 46.5 (C-7), 44.5 (C-8), 68.0 (C-9), 127.7 (C-1'), 111.0 (C-2'), 145.8 (C-3'), 143.7 (C-4'), 116.0 (C-5'), 132.9 (C-6'), 32.4 (C-7'), 38.2 (C-8'), 65.5 (C-9'), 104.4 (Glc-1), 73.6 (Glc-2), 76.5 (Glc-3), 69.9 (Glc-4), 76.5 (Glc-5), 63.7 (Glc-6), 55.1(3-OCH_3), 55.0 (3'-OCH_3).

2.4.11 Rhodiooctanoside (11)

White powder, $C_{19}H_{36}O_{10}$, APCI-MS *m/z*: 442 $[M+H_2O]^+$; UV λ_{max}: 202 nm; ^1H NMR (CD_3OD, 400MHz) δ 3.85 (1H, *m*, H-1a), 3.58 (1H, *m*, H-1b), 1.20-1.57 (12H, H-2 to H-7), 0.89 (3H, *t*, *J* = 5.6 Hz, H-8), 4.23 (1H, *d*, *J* = 7.6 Hz, Glc-1), 4.07 (1H, *d*, *J* = 11.2 Hz, Glc-6Ha), 3.72 (1H, *dd*, *J* = 11.4, 5.2 Hz, Glc-6Hb), 4.30 (1H, *d*, *J* = 6.8 Hz, Ara-1); ^{13}C NMR (CD_3OD, 100MHz) δ 72.1 (C-1), 33.5 (C-2), 31.3 (C-3), 30.9 (C-4), 31.1 (C-5), 27.7 (C-6), 24.2 (C-7), 14.9 (C-8), 104.9 (Glc-1), 75.6 (Glc-2), 78.5 (Glc-3), 71.5 (Glc-4), 77.4 (Glc-5), 70.0 (Glc-6), 105.7 (Ara-1), 72.9 (Ara-2), 74.7 (Ara-3), 70.0 (Ara-4), 67.2 (Ara-5).

2.4.12 Sacranoside B (12)

Brown powder, $C_{21}H_{36}O_{10}$, APCI-MS *m/z*: 466 $[M+H_2O]^+$; UV λ_{max}: 202 nm; ^1H NMR (CD_3OD, 400MHz) δ 4.26 - 4.31 (2H, *m*, H-1a, H-1b), 5.36 (1H, *t*, H-2), 2.09 (4H, *m*, H-4, H-5), 5.11 (1H, *t*, H-6), 1.60 (3H, *s*, H-8), 1.67 (3H, *s*, H-9), 1.74 (3H, *s*, H-10), 4.30 (1H, *d*, *J* = 6.4 Hz, Glc-1H), 3.17 - 3.87 (6H, Glc-2H to Glc-6H), 4.27 (1H, *d*, *J* = 8 Hz, Ara-1); ^{13}C NMR (CD_3OD, 100MHz) δ 66.9 (C-1), 123.0 (C-2), 142.4 (C-3), 33.6 (C-4), 28.2 (C-5), 125.5 (C-6), 133.4 (C-7), 18.4 (C-8), 26.5 (C-9), 24.2 (C-10), 103.6 (Glc-1), 75.1 (Glc-2), 78.4 (Glc-3), 72.0 (Glc-4), 77.2 (Glc-5), 69.9 (Glc-6), 105.6 (Ara-1), 72.9 (Ara-2), 74.7 (Ara-3), 79.8 (Ara-4), 67.1 (Ara-5).

Chapter 3 Results and Discussion

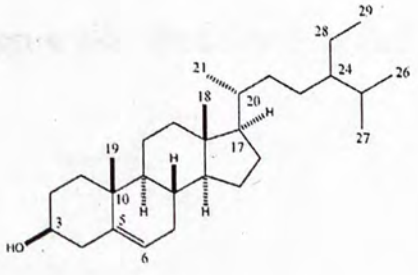
From the dried root of *Rhodiola kirilowii*, eleven compounds were isolated and purified. Compound **3** to **12** were for the first time purified from this plant species. Compound **4** and **5** were for the first time purified from this genus.

3.1 Structural Determination of the Isolated Compounds

3.1.1 Identification of β-sitosterol (1)

Compound **1** was isolated as colourless needles. Its ^{1}H and ^{13}C NMR spectra exhibited the characteristics of a phytosterol. ^{13}C NMR spectrum showed twenty-nine carbons, with one carbon being oxygenated (δ 71.8, C-3). The ^{1}H NMR spectrum displayed a characteristic proton signal of an olefinic carbon at δ 5.32 (H-6) and a proton signal at δ 3.50 (H-3α). Careful comparison of the ^{13}C NMR spectroscopic data with the literature values [61, 62], suggested that compound **1** was β-sitosterol (Figure 3-1).

Figure 3-1. Structure of β-sitosterol (**1**)



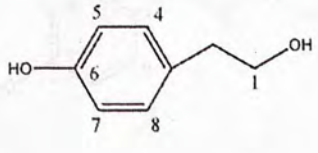
3.1.2 Identification of Tyrosol (2)

Compound **2** was isolated as colourless needles. The ^{1}H and ^{13}C NMR spectra indicated that compound **2** was a phenylethyl alcohol derivative.

The ^{1}H NMR spectrum showed two triplet signals at δ 3.67 (2H, *t*, *J* = 7.1 Hz, H-1) and 2.70 (2H, *t*, *J* = 7.1 Hz, H-2) belonging to two methylene groups. In addition, from the ^{13}C NMR spectrum, one hydroxylated methylene carbon signal was observed at δ 64.5. Therefore, the two methylene carbons were connected to each other with one being hydroxylated.

The ^{1}H NMR spectrum of **2** displayed four aromatic proton signals at δ 7.01 (2H, *d*, *J* = 8.8 Hz, H-4, 8) and 6.69 (2H, *d*, *J* = 8.3 Hz, H-5, 7). Besides, the ^{13}C NMR spectrum showed three aromatic carbon signals at δ 130.7, 116.0, and 156.5. Thus, the benzyl moiety was deduced to be with a symmetric structure, 1,4-disubstituted pattern. By careful comparison of the NMR spectroscopic data with those reported in the literature [63], compound **2** was identified to be tyrosol (Figure 3-2).

Figure 3-2. Structure of tyrosol (**2**)

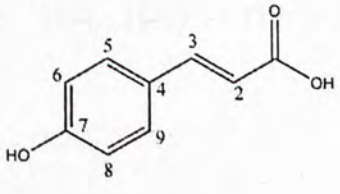


3.1.3 Identification of *trans*-Hydroxycinnamic Acid (3)

Compound **3** was isolated as colourless needles. The ^{1}H NMR spectrum displayed six proton signals. The ^{13}C NMR spectrum showed seven carbon signals included one carbonyl group (δ 171.5). By careful comparison of the NMR spectroscopic data with those reported in the literature [64], compound **3** was identified to be *trans*-hydroxycinnamic acid.

In this compound, the two olefinic protons in *trans*- configuration appeared as doublet signals at δ 6.27 (1H, *d*, *J* = 15.6 Hz, H-2) and 7.59 (1H, *d*, *J* = 15.6 Hz, H-3). The four aromatic protons signals were displayed at δ 7.43 (2H, *d*, *J* = 8.4 Hz, H-5, 9), 6.89 (2H, *d*, *J* = 8.4 Hz, H-6, 8). The ^{13}C NMR spectrum of this compound displayed a symmetric aromatic structure by the presence of carbon signals at δ131.6 (C-5, 9) and 117.3 (C-6, 8). The oxygenated aromatic carbon signal was displayed at δ 161.6 (C-7) and the two olefinic carbons showed signals at δ 116.1 (C-2) and 147.1 (C-3) (Figure 3-3).

Figure 3-3. Structure of *trans*-hydroxycinnamic acid (**3**)



3.1.4 Identification of Geranyl-β-glucopyranoside (4)

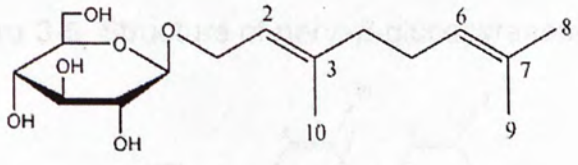
Compound **4** was isolated as brown powder. The ^{1}H NMR spectrum showed sugar proton signals and seventeen other proton signals consisted of methyl protons, methylene protons and olefinic protons. The ^{13}C NMR spectrum displayed sixteen carbon signals, including six sugar carbons and ten other carbons signals. This suggested that **4** was a monoterpene glycoside. The ^{1}H and ^{13}C NMR spectra were compared with the literature values, and the alycone monoterpene was concluded as geraniol [37].

Under this structure, the alycone of **4** consisted of two olefinic bonds, in which C_2-C_3 olefinic bond appeared in *E*- configuration. The two olefinic proton signals displayed at δ 5.37 (1H, *m*, H-2) and 5.10 (1H, *m*, H-6). There were three methyl carbons (δ 18.3, C-8; 26.4, C-9; 17.0, C-10) adjacent to olefinic carbons with proton signals at δ 1.60 (3H, *s*, H-8), 1.67 (3H, *s*, H-9), 1.75 (3H, *s*, H-10) and three methylene carbons (δ 66.8, C-1; 41.2, C-4; 27.9, C-5) adjacent to olefinic carbons with proton signals at δ 4.18 - 4.34 (2H, *m*, H-1a, H-1b), 2.0 – 2.2 (4H, *m*, H-4, H-5). The methylene carbon C-1 was oxygenated (δ 66.8).

The six sugar carbon showed characteristic signals corresponding to a glucopyranose (δ 103.2, Glc-1; 75.6, Glc-2; 78.7, Glc-3; 72.2, Glc-4; 78.5, Glc-5; 63.3, Glc-6). The glucosyl anomeric proton at δ 4.28 (1H, *d*, *J* = 8.0 Hz, Glc-1H) suggested a *β* configuration [65].

By careful comparison of the NMR spectroscopic data with those reported in the literature [66], **4** was elucidated as geranyl-*β*-glucopyranoside (Figure 3-4).

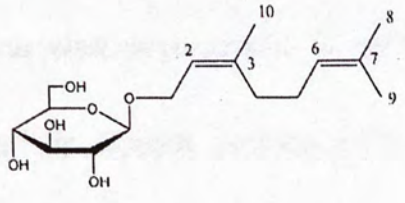
Figure 3-4. Structure of geranyl-*β*-glucopyranoside (**4**)



3.1.5 Identification of Neryl-β-glucopyranoside (5)

Compound **5** was isolated as brown powder. The NMR spectra information of **5** was similar to **4**, except that the C-10 carbon signal appeared at δ 24.2 instead of δ 17.2 as in **4**. This suggested **5** had a Z- configuration. By comparing with literature data [66], the alycone monoterpene of **5** was concluded as nerol, and **5** was elucidated as neryl-β-glucopyranoside (Figure 3-5).

Figure 3-5. Structure of neryl-β-glucopyranoside (**5**)

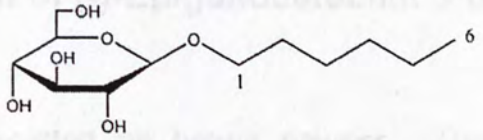


3.1.6 Identification of Hexyl β-Glucopyranoside (6)

Compound **6** was isolated as pale yellow powder. The ^{1}H NMR spectrum showed proton signals corresponding to a sugar unit and thirteen other protons consisted of ten methylene protons (δ 1.52, 2H, *t*, *d* = 7.4 Hz, H-1; 1.21 to 1.30, 8H, H-2 to H-5) and three methyl protons (δ 0.80, 3H, *t*, *d* = 6.6 Hz, H-6).

The ^{13}C, DEPT NMR spectra displayed six sugar carbons consisted of five methylene carbons (δ 69.5, C-1; 29.4, C-2; 25.7, C-3; 31.6, C-4; 22.3, C-5) and one methyl carbon (δ 13.0, C-6). The ^{13}C NMR data indicated that one of the methylene carbons was oxygenated (δ 69.5, C-1). The six sugar carbon showed characteristic signals corresponding to a glucopyranose (δ 103.0, Glc-1; 73.7, Glc-2; 76.7, Glc-3; 70.2, Glc-4; 76.5, Glc-5; 61.4, Glc-6). The glucosyl anomeric proton at δ 4.15 (1H, *d*, *J* = 7.6 Hz, Glc-1H) suggested a β configuration [65]. Based on the available evidence that there is only one methyl group, five methylene groups (one oxygenated) and a β-glucose unit with anomeric carbon at δ 103.0, compound **6** was elucidated to be hexyl β-glucopyranoside [67] (Figure 3-6).

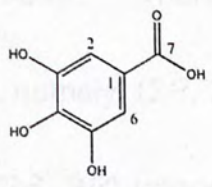
Figure 3-6. Structure of hexyl β-glucopyranoside (**6**)



3.1.7 Identification of Gallic Acid (7)

Compound **7** was isolated as white powder. The ^{1}H and ^{13}C NMR spectrum showed that compound **7** consisted of an aromatic ring and a carbonyl carbon. The ^{1}H NMR data suggested that the aromatic ring was tetra-substituted with two aromatic protons (δ 7.07, *s*, H-2, H-6). The ^{13}C spectrum displayed one carbonyl carbon signal at δ 170.1 (C-7) and four aromatic carbon signals at δ 146.2 (C-3, 5), 139.3 (C-4), 121.8 (C-1) and 110.2 (C-2, C-6). By detailed comparison of the NMR spectroscopic data with those reported in the literatures [30], compound **7** was concluded to be gallic acid (Figure 3-7).

Figure 3-7. Structure of gallic acid (**7**)



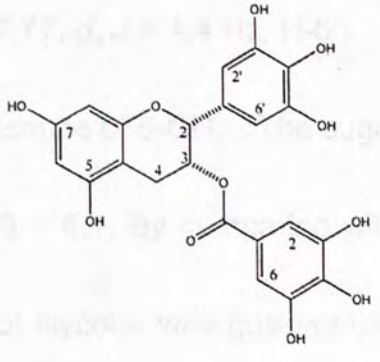
3.1.8 Identification of (-)-Epigallocatechin 3-Gallate (8)

Compound **8** was isolated as brown powder. The ^{13}C NMR spectrum displayed three characteristic signals at δ 78.6 (C-2), 69.8 (C-3), 26.9 (C-4) corresponding to a flavan-3-ol unit. Oxygenated aromatic carbon signals were observed at δ 157.6 (C-5), 157.6 (C-7), 146.1 (C-3'), 133.6 (C-4') and 146.1 (C-5'). The ^{1}H NMR spectrum showed six aromatic protons together with two methylene protons and two methine protons. The presence of a flavan-3-ol unit was also revealed by the methine proton signals (δ 4.96, 1H, *s*, H-2; 5.52, 1H, *m*, H-3) and methylene proton signals (δ 2.97, 1H, *dd, J* = 17.6, 4.4 Hz, H-4a; 2.83, 1H, *dd*, *J* = 17.8, 1.2 Hz, H-4b). A doublet signal corresponding to H-6 and H-8 of the flavan-3-ol were observed at δ 5.95 (*J* = 0.8 Hz). Two singlets were observed at δ 6.49, which were referred to the H-2' and H-6' of the flavan-3-ol. The remaining two aromatic proton signals were observed at δ 6.94. The flavan-3-ol unit was concluded to be 5,7,3',4',5'-pentahydroxyl-flavan-3-ol. There are four possible absolute configurations of C-2 and C-3, namely, (2*R*, 3*R*), (2*S*, 3*S*), (2*R*, 3*S*), or (2*S*, 3*R*). For (2*R*, 3*S*)-*trans*- or (2*S*, 3*R*)-*trans*-stereochemistry, the H-2 signal would appear as a doublet with a large coupling constant of about 6-8 Hz [68].

Since the H-2 signal of **8** showed a singlet at δ 4.96, compound **8** must be either (2*R*, 3*R*)-*cis*- or (2*S*, 3*S*)-*cis*-stereochemistry. (2*R*, 3*R*)-*cis*- and (2*S*, 3*S*)-*cis*-flavan-3-ol analogues have negative and positive specific optical rotation, respectively [68]. The $J_{2,3}$ coupling constant and optical rotation property of compound **8** (-306.7°) suggested the absolute configuration of **8** was (2*R*, 3*R*). By detailed comparison with literature values, the flavan-3-ol unit was concluded to be (-)-epigallocatechin [69].

The remaining carbon and proton signals were assignable to a gallic acid moiety based on the NMR spectroscopic data [30]. Compound **8** was therefore elucidated to be (-)-epigallocatechin 3-gallate [69, 70] (Figure 3-8).

Figure 3-8. Structure of (-)-epigallocatechin 3-gallate (**8**)

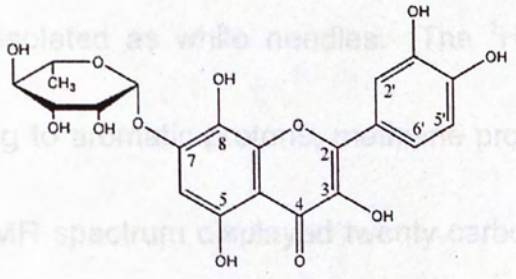


3.1.9 Identification of Rhodiolgin (9)

Compound **9** was isolated as brown powder. The ^{1}H NMR spectrum showed signals corresponding to a sugar unit and aromatic protons. The ^{13}C NMR spectrum displayed six sugar carbons and other alycone carbons. The aglycone carbon signals included one keto carbon signal (δ 177.5, C-4), two olefinic carbon signals (δ 148.5, C-2; 137.2, C-3), and ten aromatic carbon signals (five of them were oxygenated). Based on the characteristic NMR data, especially the presence of signals at δ 148.5 (C-2), 137.2 (C-3) and 177.5 (C-4), compound **9** was deduced to be a flavonol glycoside.

There were four aromatic protons present in the ^{1}H NMR spectrum, including two singlet protons (δ 6.65, H-6; 7.85, H-2') and two *ortho*-coupled protons (δ 6.89, *d*, *J* = 4.4 Hz, H-5'; 7.77, *d*, *J* = 4.4 Hz, H-6'). The chemical shift of C-4 (δ 177.5) revealed the presence of 5-OH. The sugar protons were found at δ 5.52 (1H, *s*), 1.26 and 3.5 – 4.1. By comparing with literature values, it was confirmed that the flavonol alycone was gossypetin [71]. The sugar part was determined to be rhamnose [72]. By detailed comparing NMR data with those reported in the literatures [73], the compound was identified to be gossypetin-7-O-α-rhamnopyranoside, rhodiolgin (Figure 3-9).

Figure 3-9. Structure of rhodiolgin (**9**)



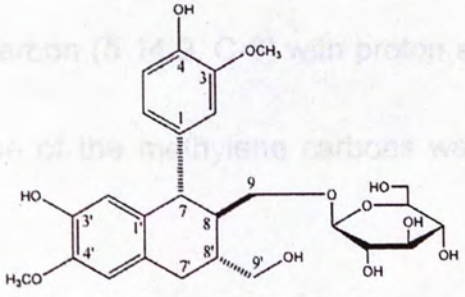
3.1.10 Identification of Isolariciresinol-9-β-glucopyranoside (10)

Compound **10** was isolated as white needles. The ^{1}H DMR data showed signals corresponding to aromatic protons, methiene protons, methylene and a glycoside. ^{13}C NMR spectrum displayed twenty carbon signals apart from those of a glycoside unit. The twenty carbon signals consisted of two aromatic rings, two methoxyl groups (δ_C 55.0, 55.1; δ_H 3.71, 3H, s; 3.72, 3H, s) and six aliphatic carbons. The six aliphatic carbons appeared to be three methines with proton signals at δ 3.96 (1H, *d*, *J* = 3.8Hz, H-7), 1.76 (1H, *m*, H-8) and 1.76 (1H, m, H-8') and three methylenes with proton signals at δ 3.39 (1H, *m*, H-9a), 3.60 (1H,*d*, *J* = 3 Hz, H-9b), 2.74 (1H, *d*, *J* = 3.6 Hz, H-7a'), 2.73 (1H, *m*, H-7b'), 3.35 (1H, m, H-9'a), and 3.63 (1H, *d*, *J* = 3 Hz, H-9'b). Two of the methylene carbons were oxygenated (δ 68.0, C-9; 65.5, C-9'). One of the aromatic rings was tetrasubstituted because two aromatic protons (δ 6.56, 1H, *s*, H-2'; 6.08, 1H, s, H-5') were observed. The other aromatic ring was trisubstituted bearing three ABX aromatic protons (δ 6.69, 1H, *s*, H-2; 6.65, 1H, *d*, *J* = 4.2 Hz, H-5; 6.53, 1H, *d*, *J* = 0.8 Hz, H-6). By comparing these characteristic signals with literature values, the alycone was deduced to be isolariciresinol [74] and compound **10** was therefore an

isolariciresinol glycoside.

The ^{13}C NMR spectrum showed characteristic signals corresponding to a glucose unit (δ 104.4, 73.6, 76.5, 69.9, 76.5, 63.7). The glucosyl anomeric proton at δ 3.96 (1H, *d*, *J*=7.6Hz, Glc-1) suggested a *β* configuration [65]. By careful comparison with literature values, compound **10** was elucidated to be isolariciresinol-9-*O*-*β*-glucopyranoside [75] (Figure 3-10).

Figure 3-10. Structure of isolariciresinol-9-O-*β*-glucopyranoside (**10**)



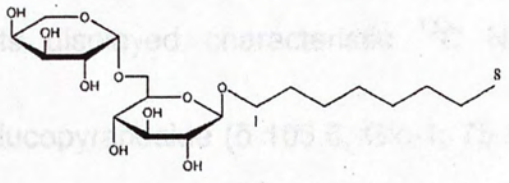
3.1.11 Identification of Rhodiooctanoside (11)

Compound **11** was isolated as white powder. The ^{1}H NMR spectrum showed signals corresponding to sugar units, methylene protons and methyl protons. The ^{13}C NMR spectrum displayed sugar carbon signals and eight other carbon signals consisting of seven methylene carbons (δ 72.1, C-1; 33.5, C-2; 31.3, C-3; 30.9, C-4; 31.1, C-5; 27.7, C-6; 24.2, C-7; 14.9, C-8) with proton signals at δ 3.85 (1H, *m*, H-1a), 3.58 (1H, *m*, H-1b), 1.20-1.57 (12H, H-2 to H-7) and one methyl carbon (δ 14.9, C-8) with proton signals at δ 0.89 (3H, *t*, *J* = 5.6 Hz, H-8). One of the methylene carbons was oxygenated (δ 72.1, C-1).

The ^{13}C NMR spectrum displayed characteristic sugar signals corresponding to a glucopyranoside (δ 104.9, Glc-1; 75.6, Glc-2; 78.5, Glc-3; 71.5, Glc-4; 77.4, Glc-5; 70.0, Glc-6) and arabinopyranoside (δ 105.7, Ara-1; 72.9, Ara-2; 74.7, Ara-3; 70.0, Ara-4, 67.2, Ara-5). The glucosyl anomeric proton at δ 4.23 (1H, *d*, *J*=7.6Hz, Glc-1) suggested a *β* configuration [65]. Moreover, the arabinosyl anomeric proton at δ 4.30 (1H, *d*, *J* = 6.8 Hz, Ara-1) revealed an α configuration [76] The presence of Glc-6 carbon signal at δ 70.0 indicated a 1→6 glycosidic linkage [77].

By careful comparison with literature values, compound **11** was elucidated to be rhodiooctanoside [76] (Figure 3-11).

Figure 3-11. Structure of rhodiooctanoside (**11**)

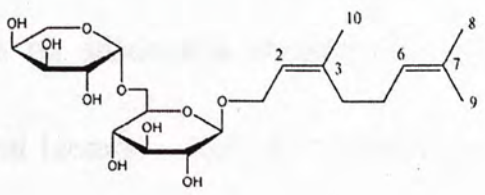


3.1.12 Identification of Sacranoside B (12)

Compound **12** was isolated as brown powder. The NMR spectra information of **12** was similar to 5, except that there was two sugar units present in **12**. The two sugar units displayed characteristic ^{13}C NMR signal patterns corresponding to a glucopyranoside (δ 103.6, Glc-1; 75.1, Glc-2; 78.4, Glc-3; 72.0, Glc-4; 77.2, Glc-5; 69.9, Glc-6) and an arabinopyranoside (δ 105.6, Ara-1; 72.9, Ara-2; 74.7, Ara-3; 79.8, Ara-4; 67.1, Ara-5). The glucosyl anomeric proton at δ 4.30 (1H, *d*, *J* = 6.4 Hz, Glc-1H) suggested a *β* configuration [65]. Moreover, the arabinosyl anomeric proton at δ 4.27 (1H, *d*, *J* = 8 Hz, Ara-1) revealed an α configuration [76] The presence of Glc-6 carbon signal at δ 69.9 indicated a 1→6 glycosidic linkage [77].

By careful comparison with literature values [78], compound **12** was elucidated to be sacranoside B (Figure 3-12).

Figure 3-12. Structure of sacranoside B (**12**)



Chapter 4 Conclusion

In this research, twelve compounds were isolated, namely, β-sitosterol, tyrosol, *trans*-hydroxycinnamic acid, sacranoside B, geranyl-β-glucopyranoside, neryl-β-glucopyranoside, rhodiolgin, rhodiooctanoside, gallic acid, (-)-epigallocatechin gallate, isolariciresinol-9-O-β-glucopyranoside, and hexyl β-glucopyranoside (Table 4-1).

Among these twelve compounds, ten compounds (*trans*-hydroxycinnamic acid, geranyl-β-glucopyranoside, neryl-β-glucopyranoside, sacranoside B, rhodiolgin, rhodiooctanoside, gallic acid, (-)-epigallocatechin gallate, isolariciresinol-9-O-β-glucopyranoside, and hexyl β-glucopyranoside) were the first time isolated and purified from this species, and geranyl-β-glucopyranoside and neryl-β-glucopyranoside were the first time being found in this genus.

Although salidroside is one of the active ingredients in Radix Et Rhizoma Rhodiolae, there was no salidroside isolated from *Rhodiola kirilowii.* This may be due to several factors. First, the regional variation in the chemical constituent may result in too low salidroside content for detection. Second, enzymatic activities of the glucosidase on salidroside during transportation

and storage may convert salidroside into tyrosol.

In this research, the twelve compounds isolated can be used for chemical fingerprinting in species identification and corresponding quality control of the herb. The discovery of new monoterpenoids from this genus would give new directions to the future pharmacological studies of the herb.

Table 4-1. Compounds isolated and purified from *Rhodiola kirilowii*

Category	Compounds
Phenylethyl derivatives	Tyrosol
Phenylpropanoids	*trans*-Hydroxycinnamic acid
Phenolic derivative	Gallic acid
Flavan-3-ol	(-)-Epigallocatechin 3-gallate
Flavonol	Rhodiolgin
Lignan	Isolariciresinol-9-O-β-glucopyranoside
Monoterpenoid	Geranyl-β-glucopyranoside, Neryl-β-glucopyranoside, Sacranoside B
Steroid	β-Sitosterol
Miscellaneous Compounds	Hexyl β-glucopyranoside, Rhodiooctanoside

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