A THESIS

FOR THE DEGREE OF MASTERS

ANTIOXIDATIVE FLAVONOIDS ISOLATED FROM *SASA QUELPAERTENSIS* NAKAI (LEAVES)

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ANTIOXIDATIVE FLAVONOIDS ISOLATED FROM *SASA QUELPAERTENSIS* NAKAI (LEAVES)

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This dissertation has been examined and approved.

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Date

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Summary

Leaves of *Sasa quelpaertensis* Nakai was extracted by 80% MeOH. Methanolic extract was suspended with water and successively partitioned with hexane, ethylacetate, *n*-BuOH and H_2O. Tyrosinase, elastase and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging tests were done to check the activity of each fractions. *n*-BuOH fraction showed good DPPH radical scavenging activity which led to the isolation of bioactive compounds from this extract. The *n*-BuOH extract was chromatographed on reversed phase silica gel column followed by Sephadex LH-20 gel to give 10 fractions of which fraction 9 was detected as single compound. The isolated pure compound was identified as a C-glycosylflavone, isoorientin by NMR spectroscopic data.

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I. Introduction

Natural product is a very general term that can be applied to virtually any type of molecules obtained from living cells for practical use. Natural product chemistry has been an important aspect of biochemical science from the beginning of the field. One of the first natural products of great interest was quinine. Penicillin is another classic example of a natural product. Natural products have inspired chemists and physicians for millennia. The rich structural diversity and complexity have prompted synthetic chemists to produce them in the laboratory, often with therapeutic applications in mind, and many drugs used today are natural products or natural-product derivatives. Coupled with improvements in approaches for natural-product isolation, characterization and synthesis, these could be opening the door to a new era in the investigation of natural products in academia and industry. In the past century, diverse classes of natural products have been isolated and characterized. These discoveries, along with the elucidation of biological and biochemical mechanisms of therapeutic action, have been central to the work of organic and medicinal chemists. Natural products have been invaluable as tools for deciphering the logic of biosynthesis and as platforms for developing front-line drugs.[1, 2] For example, between 1981 and 2002, 5% of the 1,031 new chemical entities approved as drugs by the US Food and Drug Administration (FDA) were natural products, and another 23% were natural-product-derived molecules.[3] Current estimates of the number of species of flowering plants range between 200,000 and 250,000 in some 300

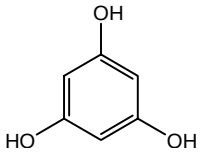
families and 10,500 genera. Despite a rapidly expanding literature on phytochemistry, only a small percentage of the total species has been examined chemically, and there is a larger field for future research.

Antioxidants are an important class of compounds which prevent the attack of destructive oxygen species in the living cell. Therefore, they play a prominent key role in food industry to extend food shelf life and also for the defense of living system against oxidative stress. Antioxidants ameliorate oxidation process by scavenging radical species, chelating catalytic metals and also acting as oxygen scavengers. In order to retard the food lipid oxidation, synthetic compounds like, BHA, BHT and TBHQ have been commonly utilized in food industry. The most popular those commercial compounds (BHA and BHT) suffer from the drawback that they have shown some carcinogenic effect on experimentally tested rats.[4] Also, highly volatile nature, thermal instability and possible mutagenic activity of those compounds create difficulty to use them in food industry. Due to unexpected bad side effects of TBHQ, it has not yet been recommended to use as a safe antioxidant in some European countries including Canada.

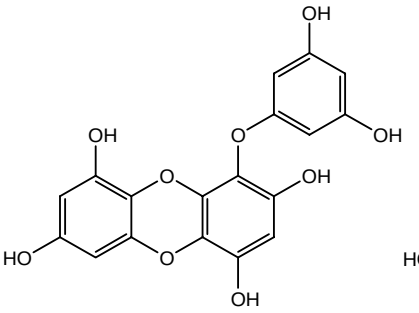
Hence, there is a very big demand in finding naturally occurring antioxidants as alternative for synthetic materials to use in food and pharmaceutical industry. After intensive experiments, scientists investigated the popular natural antioxidants like α-tocopherol and rosemary to replace commercial synthetic materials. However, those compounds also have their own limitations such as chemical instability to use them in food industry despite their high antioxidant capacity.

Therefore, still novel natural antioxidants are in high demand and higher plants and their constituents have been continuously investigated to find better and safer natural antioxidants. Among, other plant natural constituents, phenolic compounds of higher plants are highly documented for their potential antioxidant activities. Phenolic compounds therefore short-circuit a destructive chain reaction that ultimately degrades cellular membranes.

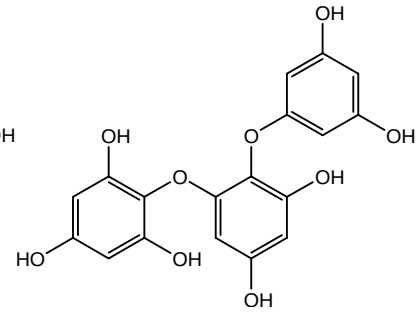
In our laboratory, continuous study is being undertaken to develop antioxidative compounds from plants collected in Jeju Island for the industrial applications. Recently we have reported isolation and characterization of antioxidative phlorotannins from *Ecklonia cava* collected offshore in Jeju Island. *E. cava*, a brown alga, is an abundant marine plant growing in water depth 5-20 m in the coast of the island. Antioxidative properties were characterized using the isolated phlorotannins, oligomeric compounds of phloroglucinol units (Fig. 1). For example, in the study of a fibroblast cell (V79-4), eckol was found to protect V79-4 cells against oxidative damage by enhancing the cellular antioxidant activity as well as modulating cellular signal pathway[5].



phloroglucinol



eckol

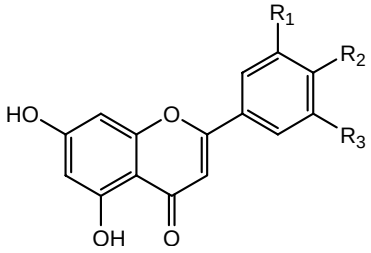


triphlorethol-A

Figure 1. Examples of antioxidative phlorotannins from *E. cava.*

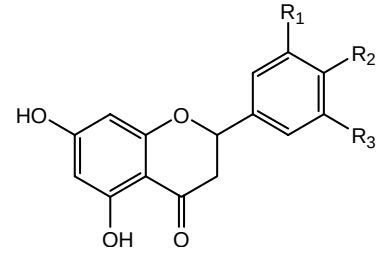
Apart from marine plants, the flavonoids are among the most efficient antioxidant molecules in terrestrial plants and more than 6,400 structures have been established. They are subdivided into various families such as flavonols, flavan-3-ols, flavones, flavanones, anthocyanins, chalcones, etc., with the flavonols, flavan-3-ols and anthocyanins being quantitatively dominant in plants.

Flavones



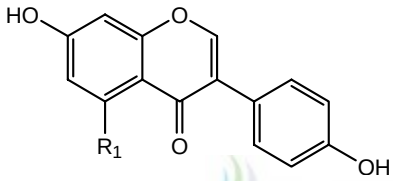
R_1= R_3= H, R_2= OH; Apigenin
R_1=R_2= OH, R_3= H; Luteolin

Flavanones



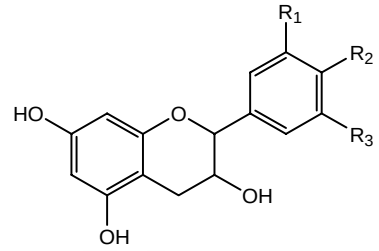
R_1=R_2= OH, R_3= H; Eriodictyol
R_1=R_3= H, R_2= OH; Naringenin

Isoflavones



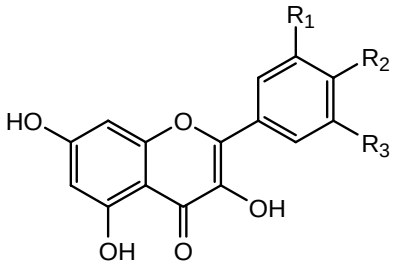
R_1= H, Daidzein
R_1= OH, Genistein

Flavanols



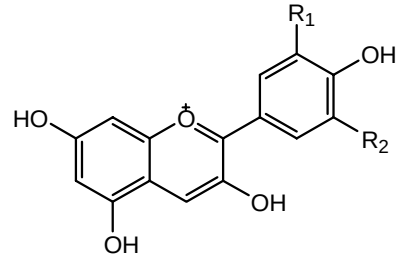
R_1= R_2= OH, R_3= H; Catechin
R_1= R_2= R_3=OH; Epigallocatechin

Flavonols



R_1=R_2= OH, R_3= H; Quercetin
R_1=R_3= H,R_2= OH; Kaempferol

Anthocyanidins



R_1=R_2=H; Pelargonidin
R_1=OH, R_2=H; Cyanidin

Figure 2. Different types of flavonoids and representative molecules

Sasa quelpaertensis Nakai is an indigenous plant of Jeju island. Jeju is an island with a variety of natural resources including thousands of varieties of marine plants in sea and terrestrial plants in mountains and land areas. Among the plant varieties in Halla Mountain *Sasa quelpaertensis* is the most abundant. Its growth habit is perennial. Each branch has 2–4 leaves and leaf-sheaths are 5–8.7 cm long with oral hairs scanty, or lacking.

Figure 3. *Sasa quelpaertensis* Nakai leaves

There is no published phytochemical report for *Sasa quelpaertensis* Nakai. In our laboratory, we are continuing to investigate bioactive compounds from plants in Jeju Island. Our interests are focused on antioxidative, anti-tyrosinase and anti-elastase compounds which are related to cosmeceutical application. This

experiment was also conducted to find bioactive compounds related to cosmeceutical industry.

II. Experimental

1. General methods

1.1 Chemicals and instruments

Analytical or laboratory grade solvents and chemicals were used in the experiments which were procured from E Merck (Germany) and also commercial grade of ethyl acetate, acetone, methanol, ethanol, chloroform and *n*-butanol were used.

All concentrations were carried out under reduced pressure using rotary vacuum evaporator at bath temperature not exceeding 40^0C.

All freeze-drying were carried out by Ilshin (Ilshin Lab Co., Ltd.) freeze-dryer. Aqueous extracts and fractions were first frozen in round-bottomed flasks in a freezer (BIOCRYOS UF8650, Biocryos Co., Ltd. Korea) at -30°C to -40^0C and finally the materials were subjected to freeze-drying operation.

NMR spectra of different extracts and fractions were recorded by using a 400 MHz NMR spectrometer (JEOL, JNM-LA400). The ^{1}H NMR spectra of pure compounds were recorded on a LAMBDA 400 MHz spectrometer using CD_3OD, DMSO-d_6. In all cases tetramethyl silane (TMS) was used as the internal reference. Two-dimensional NMR (^{1}H-^{1}H COSY, HMBC, and HMQC) spectra were obtained using standard pulse sequences.

1.2 Column chromatography

TLC plates were purchased from MERCK,Germany. 5X2.5 cm plates were used for experiment.

Capillary tubes were used for the application of samples on plates. TLC plates were developed in different solvent systems by the ascending method in glass tanks or jars.

For the location of the separated components, the plates were examined by one of the following methods:

(i) Examination under a UV light with two different wavelengths, 254 and 350 nm.

(ii) The plates were soaked in visualizing reagent ($KMnO_4$ 3 g, K_2CO_3 20 g, NaOH 0.25 g, H_2O 300 mL).

Glass columns of different sizes varying from large glass tubes (90-cm x 10 cm, i.d) to burette like tube were used. For column chromatographic separation, column grade silica gel G-60 (63-230 mesh, particle size 0.04-0.063 mm, ASTM, MERCK) was used as stationary phase.

The column was packed with silica gel slurry using the lowest polar solvent. The packed column was equilibrated with two or three column volumes of mobile phase. Then the sample was introduced on the column bed and solvents with varying polarity (hexane to methanol) were used to elute the sample.

The required amount of Sephadex LH-20 gel (particle size 25-100 μm, Amersham Biosciences, Sweden) was suspended in water for two hours. The gel was degassed (30 minutes). The column was

conditioned with three column volume of water followed by methanol. The column was then ready for sample application.

The crude extract or sub-fraction thereof was applied into the column either as a solution or in ground form. To apply the sample in powder form the sample was dissolved in a particular solvent or a mixture of solvents and silica gel (sample: gel, 1:2-3 w/w) was added to the sample solution and the mixture was evaporated to dryness. The dried material was ground thoroughly in a mortar to make fine powder and the powder was then applied into the column.

To apply the sample in the form of solution it was dissolved in a minimum volume of the column equilibrating solvent and was applied into the column. After application of the sample, some cotton or fresh silica gel was placed on the top of the silica gel bed so that the surface of the bed was not affected during solvent application.

After application of the sample, the column was eluted with the equilibrating solvent and the polarity of the mobile phase was increased gradually by adding more polar solvent such as dichloromethane, ethyl acetate, and methanol. The eluted samples were collected in conical flasks or test tubes and the fractions were monitored by using TLC profiles. Fractions were combined on the basis of their R_f values. The fractions were also combined by evaluating of their low-resolution ^{1}H-NMR spectra where applicable.

2. Biological activity tests

2.1 DPPH photometric assay

The method used by Luciana[6] was adopted with suitable modifications. Sample stock solutions were diluted to final concentrations of 200, 100, 50 and 10 μg/mL, in 70% ethanol. 0.5 mL of a 0.2 mM DPPH ethanol solution was added to 1.0 mL of sample solutions of different concentrations, and shaken well enough by vortex. After 10 min the absorbance values were measured at 525 nm and converted into the percentage antoxidant activity (AA) using the following formula:

$$AA\% = [1- (Abs_{sample} – Abs_{blank}) / Abs_{control}] X 100$$

70% ethanol (0.5 mL) plus plant extract solution (1.0mL) was used as a blank. DPPH solution (0.5 mL; 0.2 mM) plus ethanol (1.0 mL) was used as a negative control. The positive controls were those using the standard solutions.

2.2 Tyrosinase inhibition assay

Tyrosinase activity is generally determined by spectrophotometry. The procedure followed that described by Vanni[7] with suitable modification. The test reaction mixture was prepared by adding total 1.0 mL sample solution [with variable amount of 0.1M phosphate buffer (pH 6.8) for different concentrations], to which 2100 unit/mL of mushroom tyrosinase had been added, to 0.5 mL of 0.1M L-tyrosine. The test mixture (1.5 mL) was incubated for 10 min at 37^0C and the absorption at 480 nm was measured. The same mixture but without the plant extract was used as the control.

The percent inhibition of tyrosinase activity was calculated as follows:

$$\% \text{ inhibition} = [1- (Abs_{sample} - Abs_{blank}) / Abs_{control}] \text{ X } 100$$

2.3 Assay for elastase activity

Procine pancreatic elastase was assayed spectrophotometrically by the modified method of James,[8] using [succ-ala-ala-ala-*p*-nitroanilide] as the substrate, and monitoring the release of *p*-nitroanilide for 10 min at 25^0C. The amount of *p*-nitroanilide was determined by measuring the absorbance at 410 nm. The reaction mixture contained: 0.2 M Tris-HCl buffer (pH 8.0), 100 μg/mL elastase, 4 mM succ-ala-ala-ala- *p*-nitroanilide as substrate and sample (dissolved in 70% ethanol). Absorbance was measured after incubation of sample for 10 min at 25^0C. Blanks contained all the components except the enzyme.

The percentage inhibition was calculated as:

$$\text{Inhibition}(\%) = \frac{[(\text{Control-Control}_{blank}) - (\text{Sample-Sample}_{blank})]}{(\text{Control} - \text{Control}_{blank})} \text{X}100$$

3. Isolation of the compound

3.1 Plant materials

Sasa quelpaertensis Nakai (leaves) was collected on 24th Sep, 2004, from the Halla Mountain, Jeju-do, Korea. The fresh leaves

were taken into laboratory and cut into the small pieces and were air-dried. The dried leaves were used for extraction.

3.2 Solvent extraction and fractionation

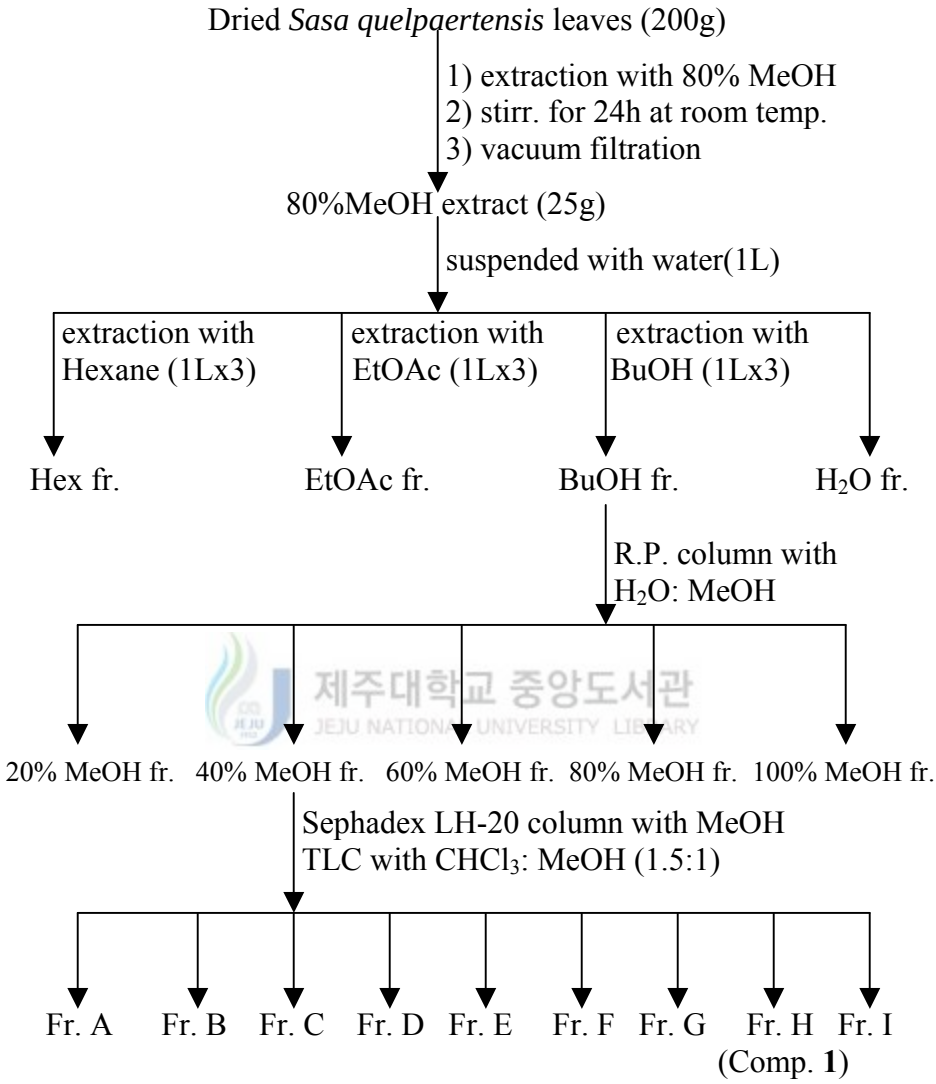
The powder of the leaves of *Sasa quelpaertensis* Nakai was extracted with 80% methanol in glass jar at room temperature for three days by changing the solvent in 24 hours. The methanol extract (10 L) was filtered by Buchner funnel and was evaporated.

The methanol extract (25 g) was suspended in water and partitioned between hexane and water. The aqueous fraction was suspended in water and partitioned between ethylacetate and water. After that the aqueous fraction was again suspended in water and partitioned between *n*-butanol and water.

3.3 Isolation procedure

200 g of dried leaves were taken and extracted three times for 24 hours in 80% MeOH at room temperature. The MeOH extract was evaporated under reduced pressure to dryness. This dry extract was suspended in water and partitioned with hexane, ethylacetae and *n*-butanol respectively. Partitionation was done thrice for each solvent. *n*-butanol extract was chromatographed on reversed-phase column using decreasing polarity trend of water-methanol solvent system. The eluted samples were collected in 5 conical flasks and then evaporated for drying. The 40% MeOH fraction was dissolved in minimum volume of methanol to get clear solution. The sample solution was then applied in the preconditioned Sephadex LH-20 column. The applied sample was eluted using methanol solvent. The eluted samples were collected in

test tubes. According to the TLC [$CHCl_3$: MeOH (1.5:1)] pattern ten different fractions were obtained. The ninth fraction (Fr. H) showed single spot on TLC plate with visualizing agent.



Scheme1. Isolation scheme of compound **1**

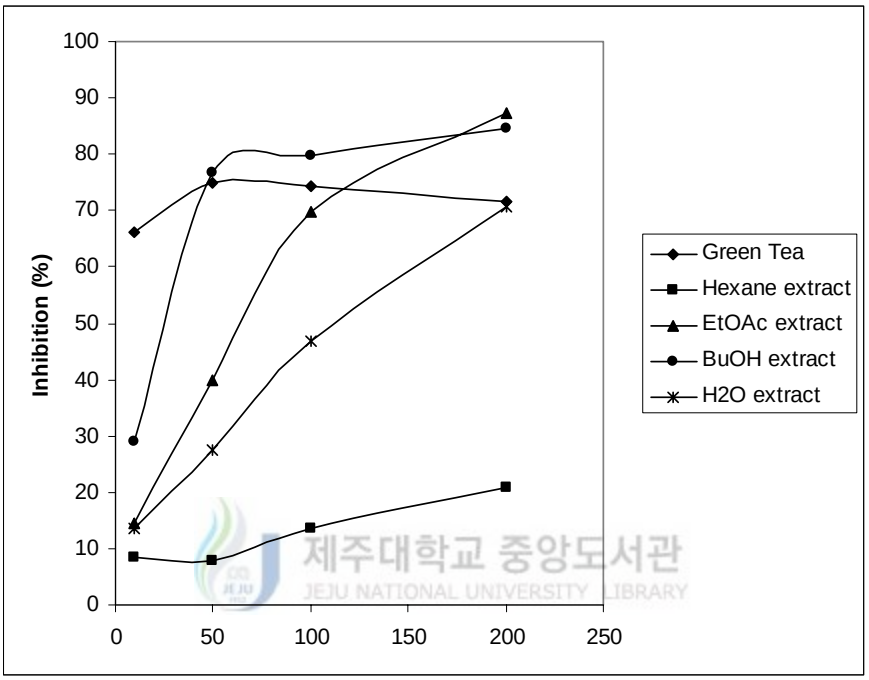
III. Results and discussion

1. Preparation of solvent fractions

The powder of the *Sasa quelpaertensis* was extracted with 80% methanol and 25 g methanol extract was obtained. The methanol extract was partitioned between hexane and water. The aqueous fraction was further partitioned with ethylacetate, *n*-butanol and aqueous part.

2. Biological activity tests of the fractions

2.1 DPPH screening



Concentration (μg/mL)

Figure 4. DPPH radical scavenging activity of various extracts

DPPH radical scavenging test was done for various extracts of *Sasa quelpaertensis* leaves. The *n*-Butanol extract have higher scavenging activity compared to other extracts, showing dose-dependent behavior. This extract also showed remarkably higher activity than the positive control, green tea except the lower concentration at 10μg/mL. All the extracts other than hexane extract showed good scavenging activity (Fig. 4).

2.2 Tyrosinase inhibition test

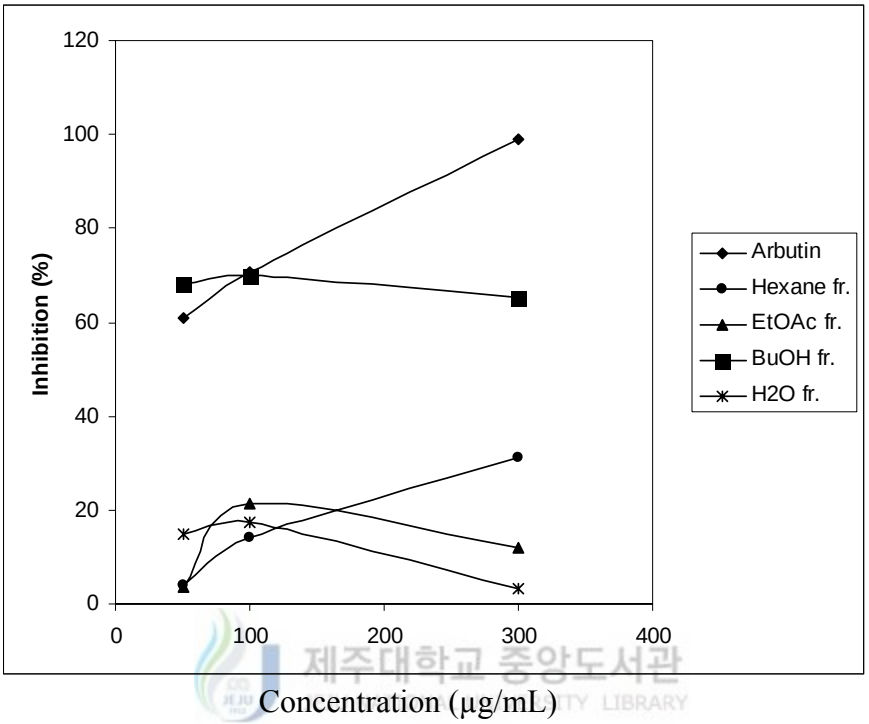


Figure 5. Tyrosinase inhibition activity of various extracts

In the tyrosinase inhibition test *n*-butanol extract showed comparatively better activity than other extracts though it was dose-independent. EtOAc and H_2O extract had decreasing inhibition trend at higher concentration (300μg/mL) which is anomalous with standard and other extracts (Fig. 5).

2.3 Elastase inhibition test

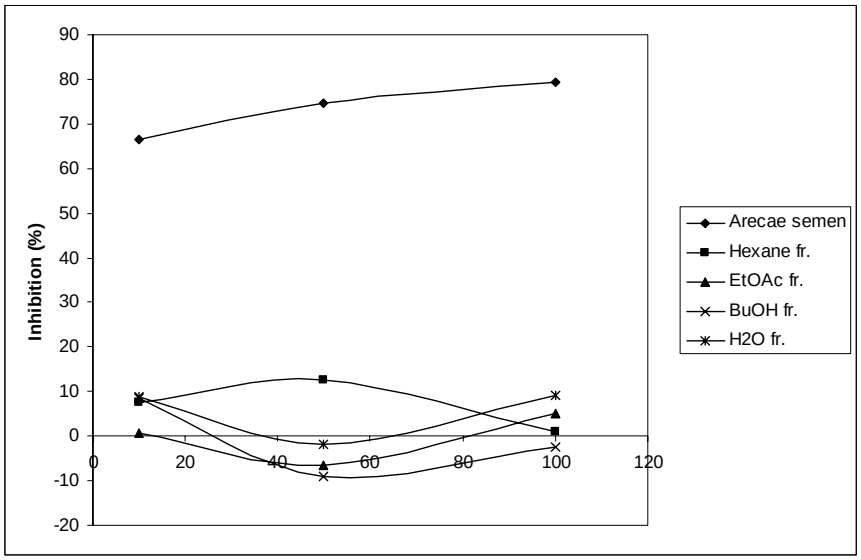


Figure 6. Elastase activity of various extracts

All the fractions obtained by solvent partition of 80% MeOH extract showed almost no activity in elastase activity assay (Fig. 6).

3. Characterization of compound 1 as Isoorientin

3.1 Structural identification of compound 1

n-Butanol fraction of methanol extract of *Sasa quelpaertensis* (leaves) was tested for tyrosinase, elastase and DPPH radical scavenging activity by our research group. It had strong free radical scavenging activity. The *n*-butanol part was was treated with reversed phase silica gel column using H_2O: MeOH (100:0 -0:100) system of decreasing polarity. 40% methanol fraction was treated with Sephadex LH-20 gel column to give ten fractions by using MeOH as eluent. The ninth fraction (Fr. H) which was 87.2 mg gave single spot on TLC plate which gave compound **1**.

This compound was obtained as a yellow amorphous solid. It was soluble in dimethyl sulfoxide. The ^{1}H NMR of this compound showed signals at the aromatic and sugar regions.

The ^{1}H NMR spectrum (Table 1) showed five aromatic protons, two singlets at δ 6.66 (s, H-3) and 6.46 (s, H-8); three signals at δ 6.86 (d, J = 8.3Hz), 7.39 (dd, J = 1.6, 8.3 Hz) and 7.42 (d, J = 1.6Hz). It also showed signals for hexose moiety. The ^{13}C NMR spectrum (Table 1) showed the presence of 21 carbon atoms in the molecule. Out of these six carbon signal (δ 73.1, 70.6, 78.9, 70.2, 81.5, 61.5) were C-glycoside group corresponding to a hexose moiety, fifteen carbon signals (163.7, 102.7, 181.8, 160.7, 108.9, 163.6, 93.5, 156.2, 103.2, 121.3, 113.2, 145.8, 149.9, 116.0, 118.9) for aglycone moiety corresponding to a flavone nucleus.

Table 1. ^{1}H and ^{13}C NMR data for compound **1** (400 MHz, **δ** values in DMSO-d_6)

Position	δ_H (ppm)	δ_C (ppm)
2		163.7
3	6.66 s	102.7
4		181.8
5		160.7
6		108.9
7		163.6
8	6.46 s	93.5
9		156.2
10		103.2
1'		121.3
2'	7.42 (d, *J* = 1.6 Hz)	113.2
3'		145.8
4'		149.9
5'	6.86 (d, *J* = 8.3 Hz)	116.0
6'	7.39 (dd, *J* = 2, 8.8 Hz)	118.9
1"	4.57 (d, *J* = 9.8 Hz)	73.1
2"	3.12 (t, *J* = 9.2 Hz)	70.6
3"	3.21 (t, *J* = 8.4 Hz)	78.9
4"	4.05 (t, *J* = 9.1 Hz)	70.2
5"	3.17 (m)	81.5
6"	3.67 (d, *J* = 10.7 Hz)	61.5
	3.41 (dd, *J* = 5.6,11.2 Hz)	

Table 2. HMBC (Heteronuclear Multiple Bond Correlation) spectral data of the compound **1**

^{1}H (δ_H)	Correlated ^{13}C (δ_C)
6.65 (H-3)	103.23 (C-10), 163.66 (C-2)
6.46 (H-8)	108.90 (C-6) , 156.21 (C-9), 103.23 (C-10)
7.39 (H-2')	149.88 (C-4'), 118.94 (C-6')
6.86 (H-5')	121.28 (C-1'), 145.80 (C-3')
4.57 (H-1")	108.90 (C-6), 163.58 (C-7)

The degree of protonation of each atom was determined by DEPT experiments, while HMQC spectrum allowed the complete correlation of the protonated carbon resonances with those of the ^{1}H spectrum. The ^{1}H-^{1}H COSY also confirmed the assignments of the protons. In the HMBC spectrum (Table 2) the anomeric protons of the glucose unit (δ 4.57 H-1") showed a ^{2}J correlation to the quaternary carbon (δ 108.9, C-6) and ^{3}J correlation to the oxymethine carbon (δ 163.6, C-7 and 160.7, C-5) of the flavone which confirmed the attachment of the C-glycosidation at position C-6 (Figure 8). On the basis of this data and comparison with the literature[9] compound **1** was identified as isoorientin (Figure7).

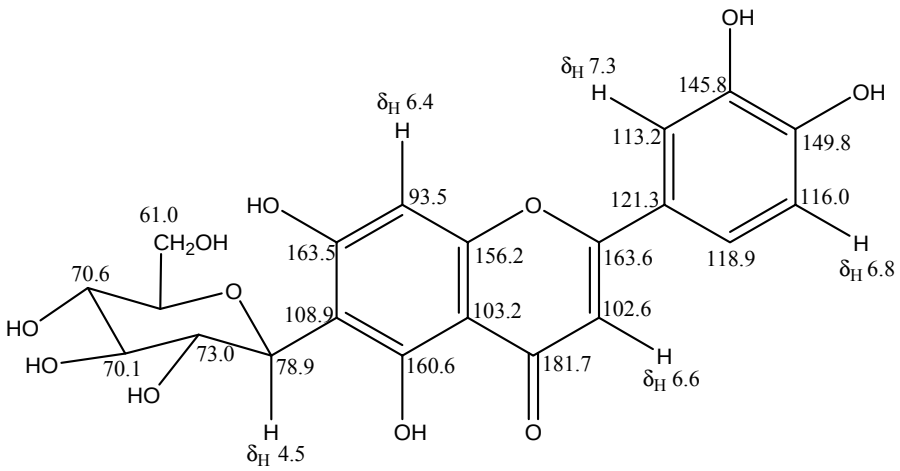


Figure 7. Chemical shift (δ) assignment of compound **1**(isoorientin)

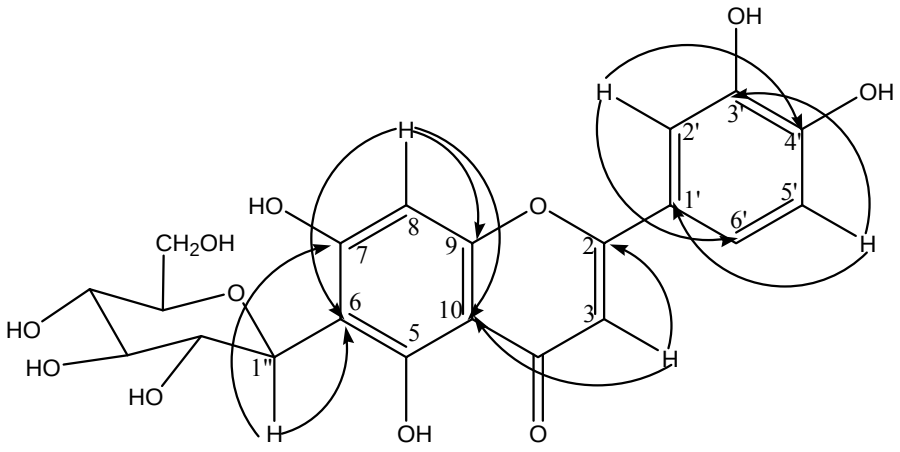


Figure 8. HMBC correlation of isoorientin

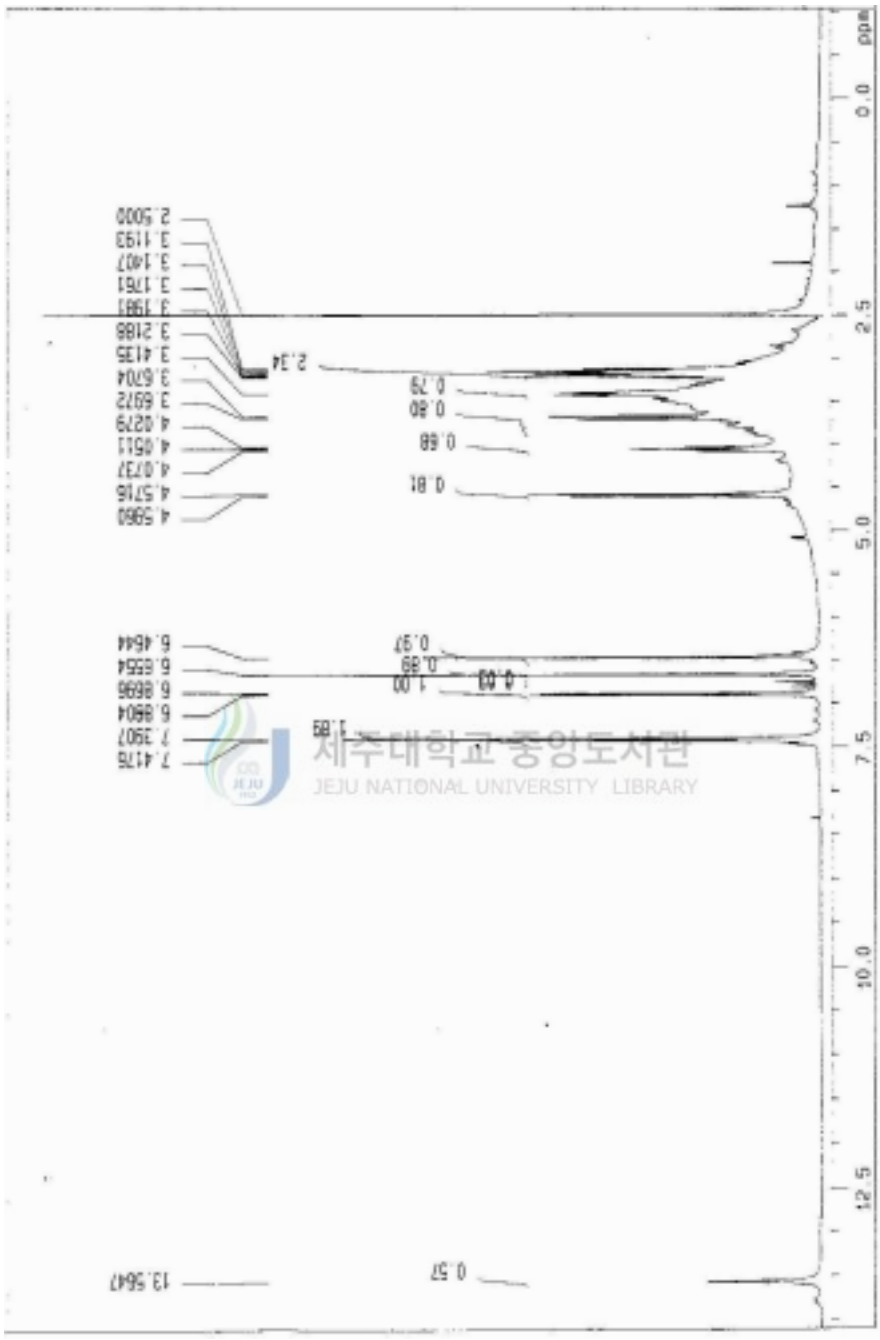


Figure 9. ^{1}H NMR spectrum of compound **1**

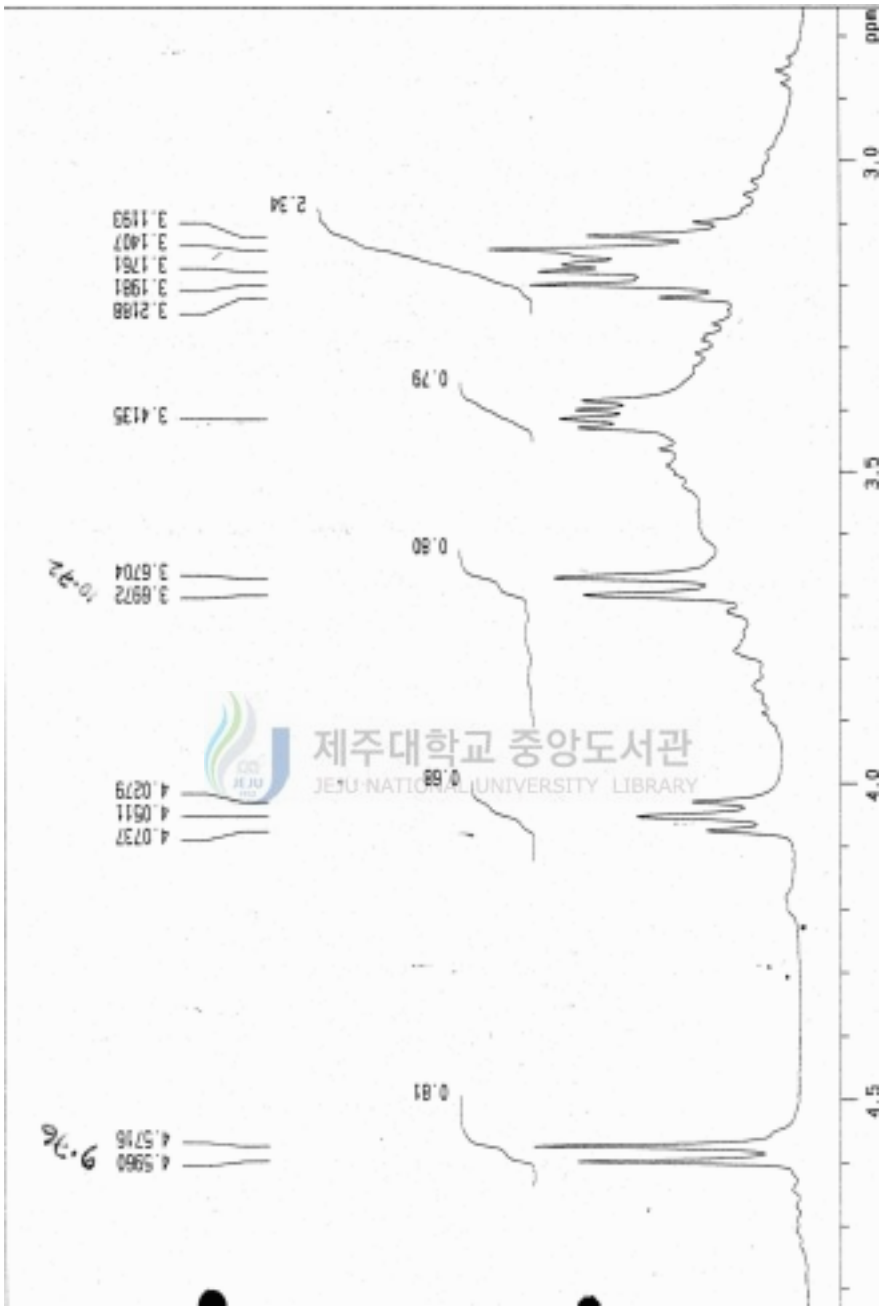


Figure 10. ^{1}H NMR spectrum of compound **1** (expanded)

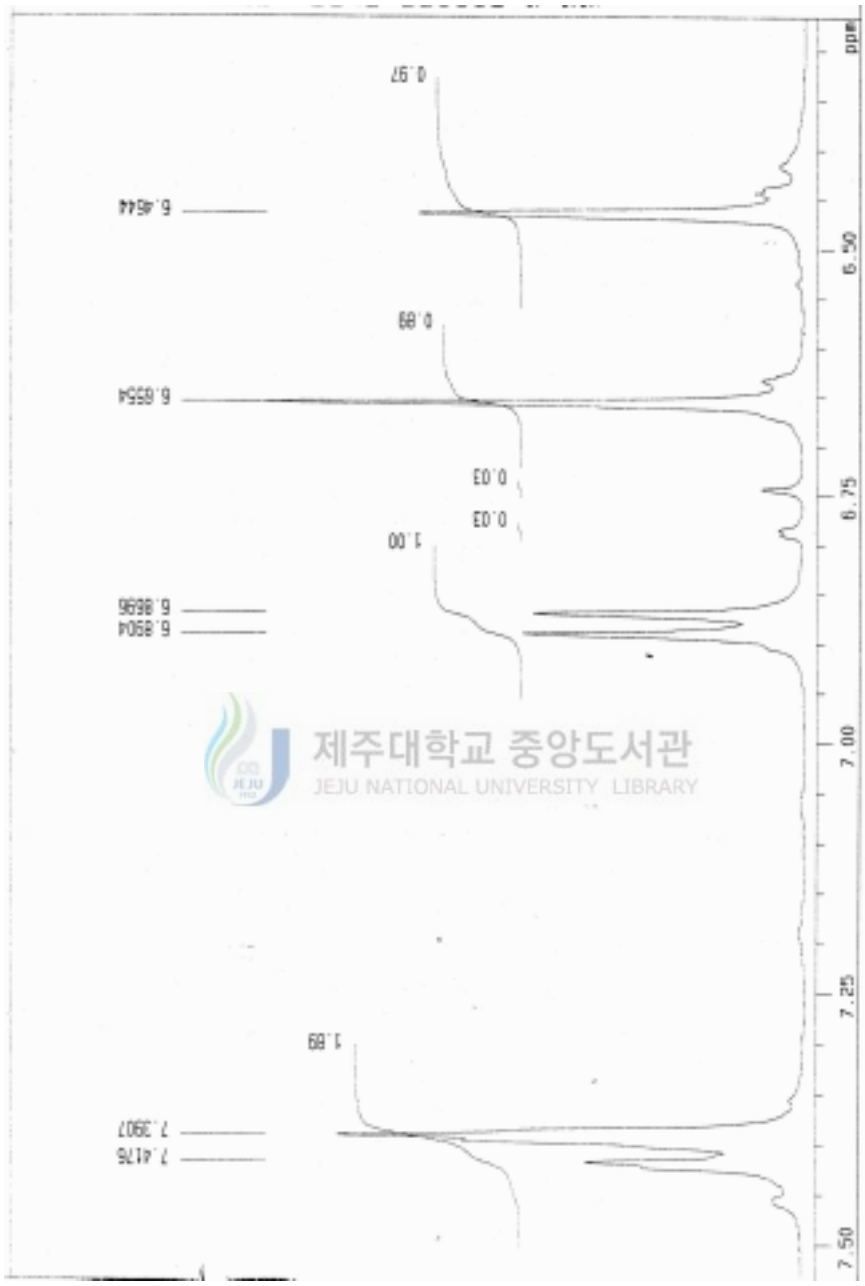


Figure 11. ^{1}H NMR spectrum of compound **1** (expanded)

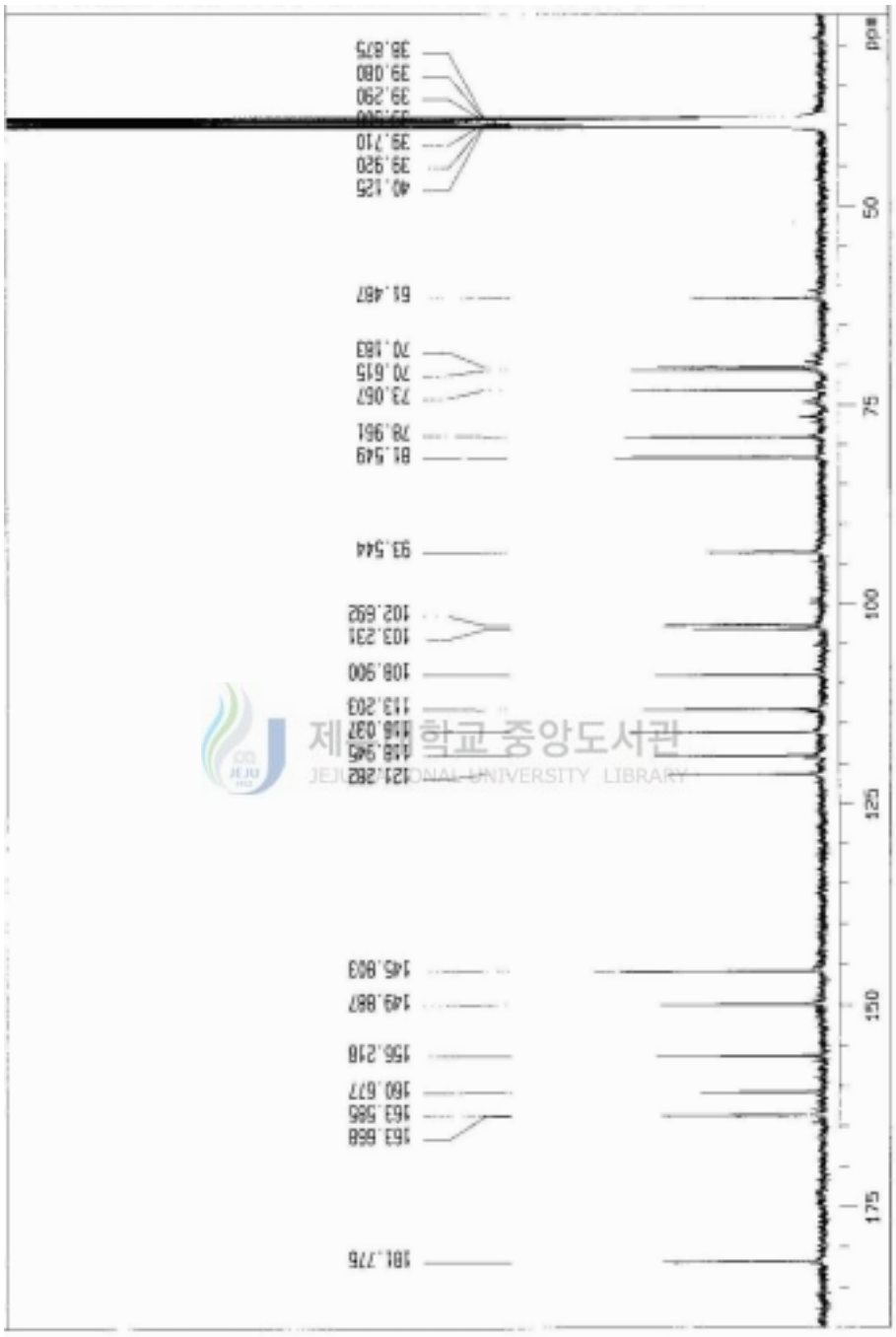


Figure 12. ^{13}C NMR spectrum of compound **1**

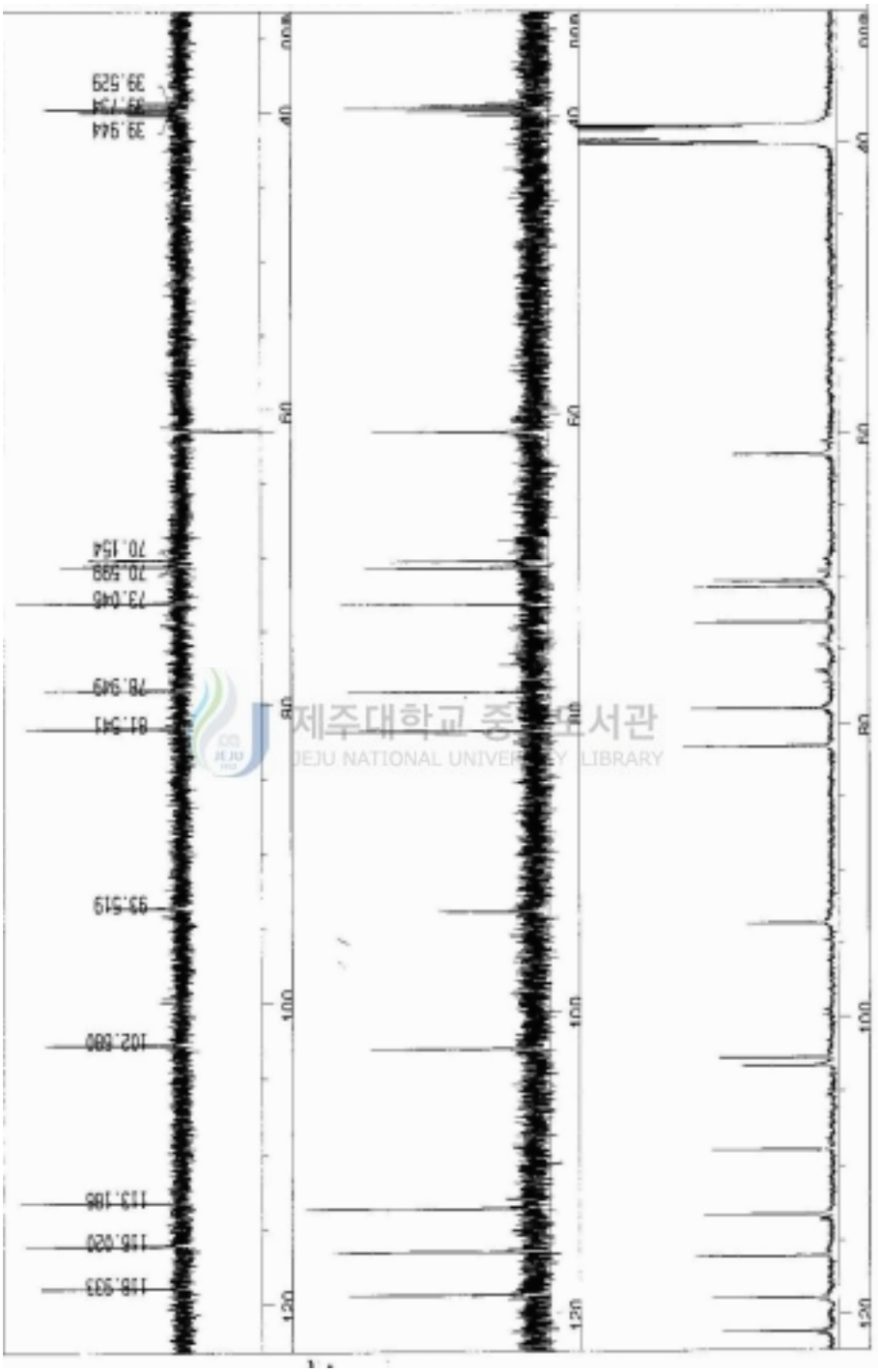


Figure 13. DEPT data of compound **1**

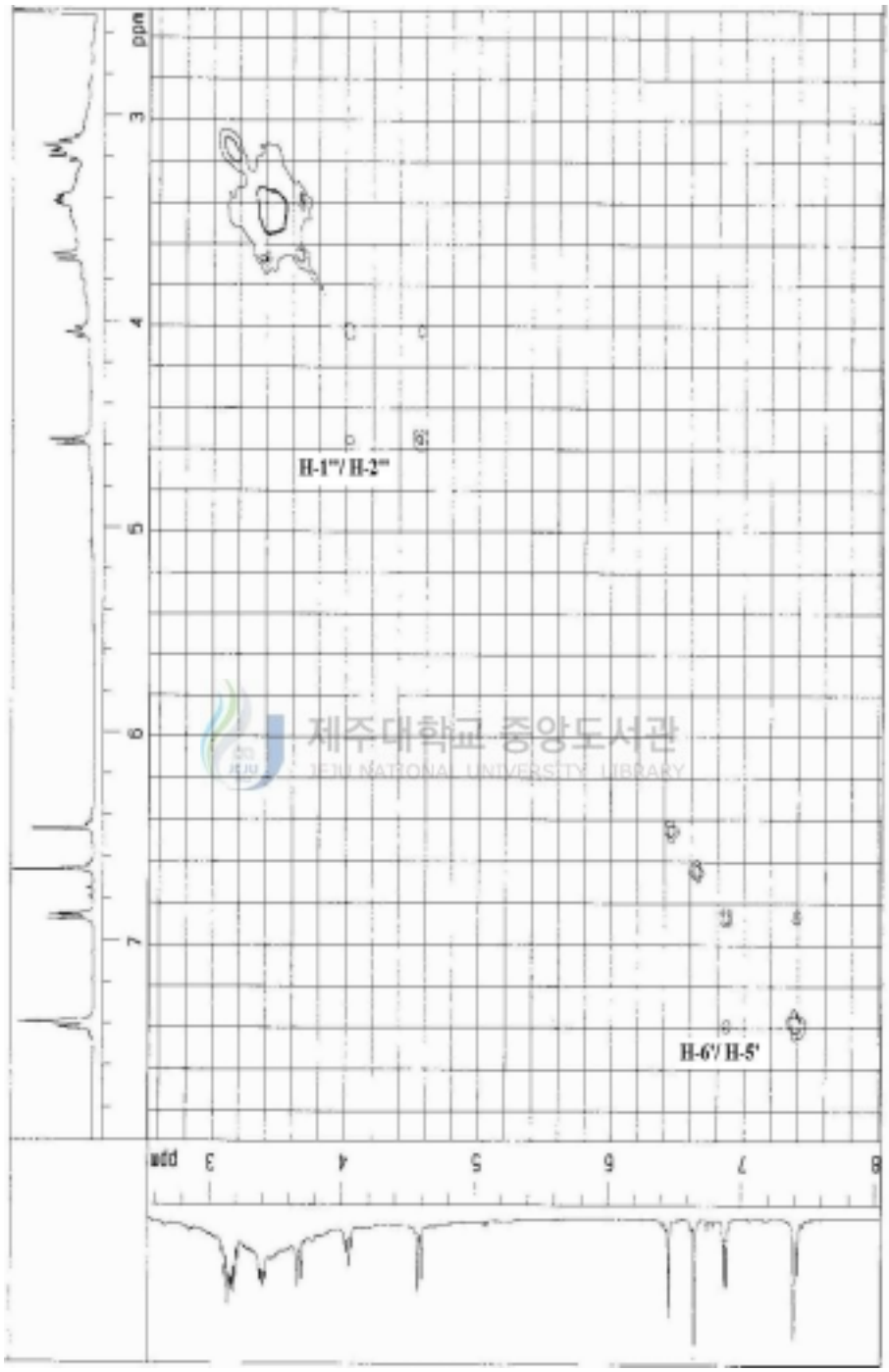


Figure 14. ^{1}H - ^{1}H COSY spectrum of compound **1**

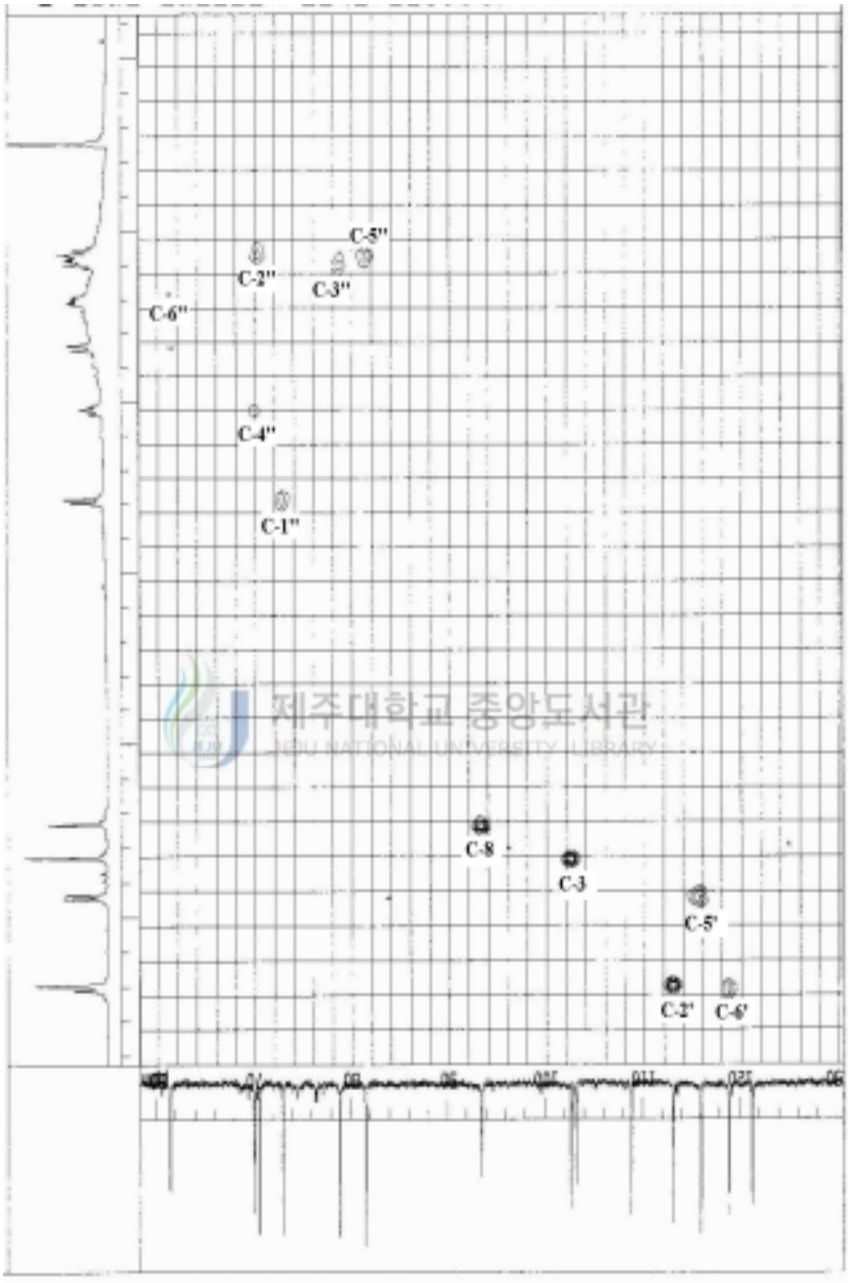


Figure 15. HMQC spectrum of compound **1**

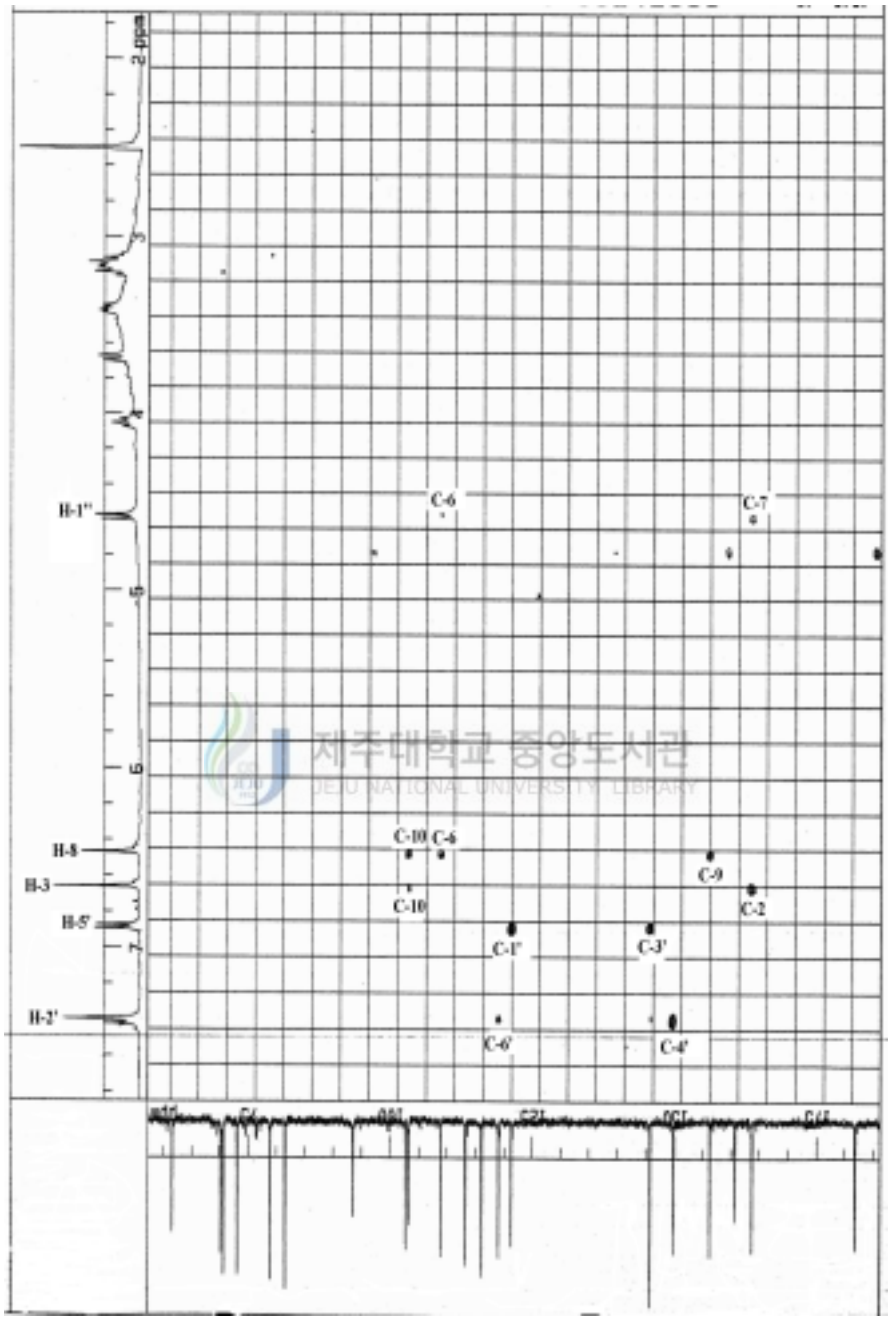


Figure 16. HMBC spectrum of compound **1**

3.2 Biological activity tests of isoorientin

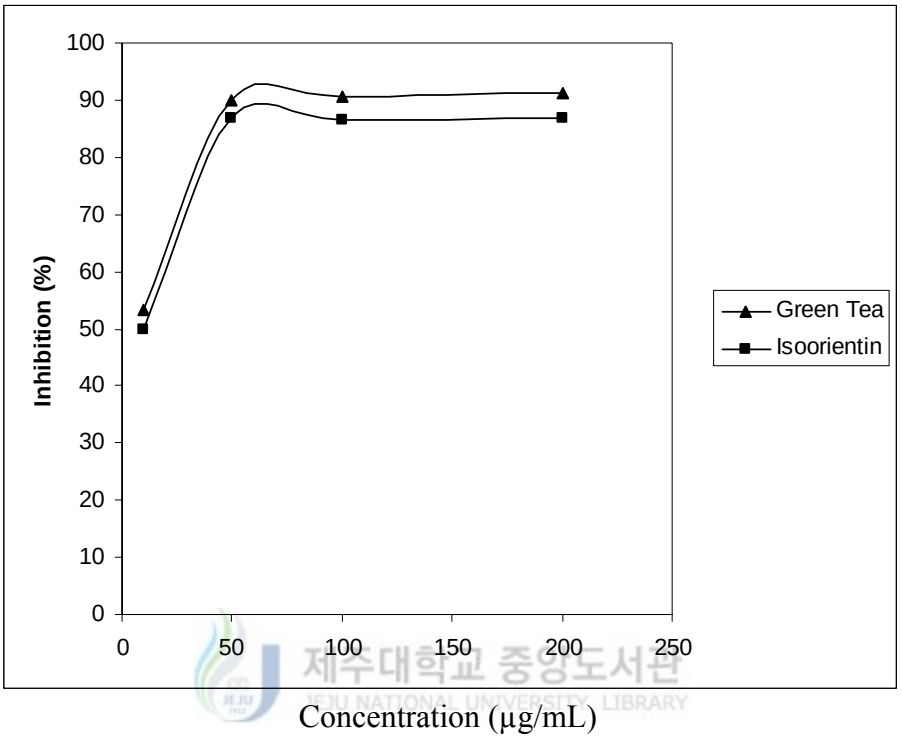


Figure 17. DPPH radical scavenging activity of isoorientin

DPPH radical scavenging test was done for isoorientin. It showed high scavenging activity as green tea with dose-dependent manner. Its activity was remarkable at low concentration (10μg/mL) also which is about 50% inhibition, almost same like positive control.

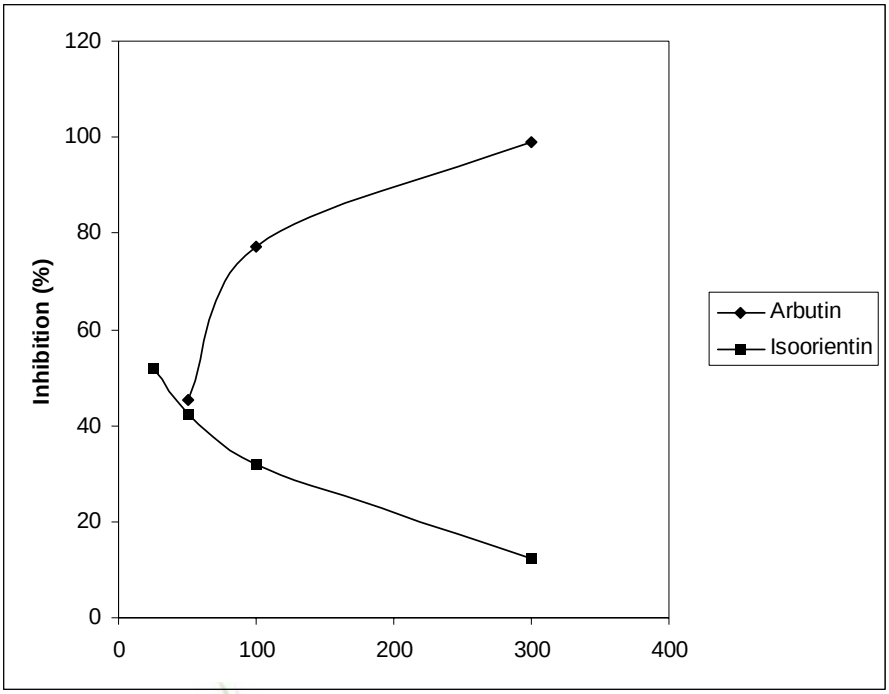


Figure 18. Tyrosinase inhibition activity of isoorientin

In the tyrosinase inhibition test Isoorientin showed decreasing inhibition trend at higher concentrations which require further study and experiment for better understanding.

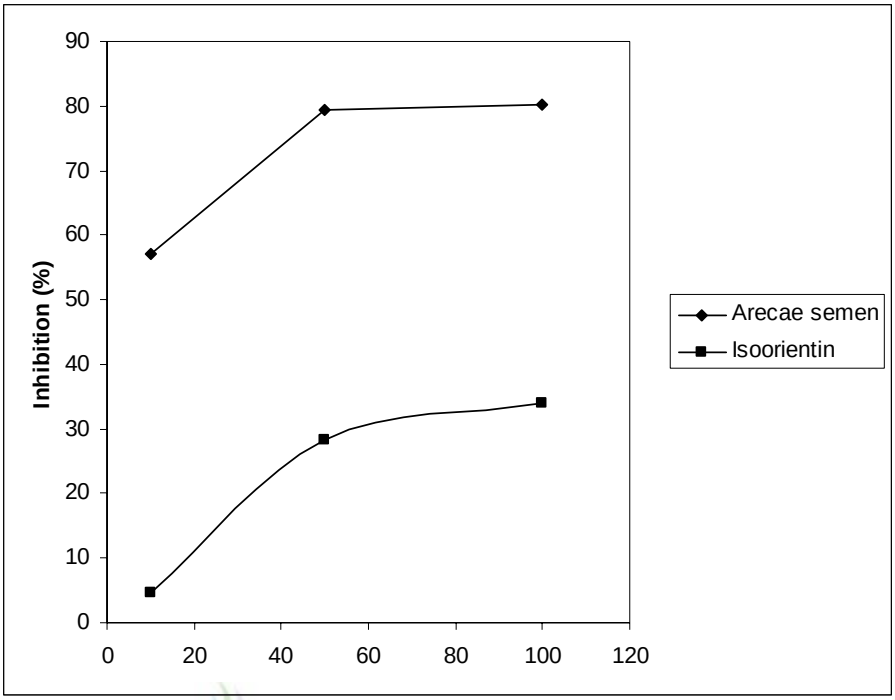


Figure 19. Elastase activity of isoorientin

Isoorientin showed low elastase inhibition activity.

According to Halliwell and Gutteridge[10] mechanisms of antioxidant action can include (1) suppressing reactive oxygen species formation either by inhibition of enzymes or chelating trace elements involved in free radical production; (2) scavenging reactive oxygen species; and (3) upregulating or protecting antioxidant defenses. Flavonoids have been identified as fulfilling most of the criteria described above. Thus, their effects are twofold.

First, flavonoids inhibit the enzymes responsible for superoxide anion production, such as xanthine oxidase[11] and protein kinaseC.[12] Flavonoids have been also shown to inhibit cyclooxygenase, lipoxygenase, microsomal monooxygenase, glutathione *S*-transferase, mitochondrial succinoxidase, and NADH oxidase, all involved in reactive oxygen species generation.[13,14] A number of flavonoids efficiently chelate trace metals, which play an important role in oxygen metabolism. Free iron and copper are potential enhancers of reactive oxygen species formation, as exemplified by the reduction of hydrogen peroxide with generation of the highly aggressive hydroxyl radical,

$$H_2O_2 + Fe^{2+} (Cu^{+}) \longrightarrow \cdot OH + OH^{-} + Fe^{3+} (Cu^{2+})$$

or by the copper-mediated LDL (low-density lipoprotein) oxidation, where LH represents LDL.

Nevertheless, it has to be remembered that these metal ions are essential for many physiological functions, as constituents of hemoproteins and cofactors of different enzymes, including those involved (iron for catalase, copper for ceruloplasmin and *Cu,Zn*-superoxide dismutase) in the antioxidant defense.[15] The proposed

binding sites for trace metals to flavonoids are the catechol moiety in ring B, the 3-hydroxyl, 4-oxo groups in the heterocyclic ring, and the 4-oxo, 5-hydroxyl groups between the heterocyclic and the A rings.

Second, due to their lower redox potentials ($0.23 < E_7 < 0.75$ V),[16] flavonoids (Fl-OH) are thermodynamically able to reduce highly oxidizing free radicals with redox potentials in the range 2.13-1.0 V,[17] such as superoxide, peroxyl, alkoxyl, and hydroxyl radicals by hydrogen atom donation:

$$\text{Fl-OH} + \text{R}\cdot \longrightarrow \text{Fl-O}\cdot + \text{RH}$$

where R· represents superoxide anion, peroxyl, alkoxyl, and hydroxyl radicals.[18-20] The aroxyl radical (Fl-O·) may react with a second radical, acquiring a stable quinone structure. The aroxyl radicals could interact with oxygen, generating quinones and superoxide anion, rather than terminating chain reactions. The last reaction may take place in the presence of high levels of transient metal ions and is responsible for the undesired prooxidant effect of flavonoids.[21] Thus, the overall capacity of flavonoids to act as antioxidants depends not only on the redox potential of the couple Fl-O·/Fl-OH but also on possible side reactions of the aroxyl radical. Scavenging of superoxide is particularly important, because this radical is ubiquitous in aerobic cells and, despite its mild reactivity, is a potential precursor of the aggressive hydroxyl radical in the Fenton and Haber-Weiss reactions.[22] Besides scavenging, flavonoids may stabilize free radicals involved in oxidative processes by complexing with them. [23]

IV. Conclusion

Sasa species (Gramineae) are widely distributed throughout Japan, the Russian part of the Kuril Islands and the southern part of Sakhalin Island. Extracts from *Sasa* plants are used as stimulation drinks in Japan, China and Estonia.[24] *Sasa albomarginata* has been described to have antibacterial and anticancer activity.[25-27] It is also found that lignan prepared from bamboo leaves has an anticancer effect.[28] In addition, polysaccharides from *Sasa* sp. are reported to have an antitumor activity.[29] Recently, two polysaccharide preparations, GK1 and GK2, have been isolated from the leaves of the bamboo grass *Sasa kurilensis* (Fr. Et Sar.). Both preparations negatively affect the growth of Sarcoma 180 implanted in mice and no signs of toxicity have been found: doses of 180 mg/kg were administered ten times to animals with no signs of toxicity at all.[30] Different kinds of compounds such as friedelin, glutinol, isoorientin, tricin, tricin 7-O-β-D-glucopyranoside, luteolin 6-C-α-L-arabinoyranoside has also been isolated from *Sasa borelis*.[31]

For this work, leaves of *Sasa quelpaertensis* was used. A C-glycosyl flavonoid, isoorientin was isolated from this plant. This compound was also isolated from *Alliaria petiolata*.[9] Among three types of bioactivity (antioxidant, tyrosinase, elastase), it showed good DPPH radical scavenging activity which is comparable to well known DPPH radical scavenger green tea. For the use of isoorientin as antioxidant compound further studies and experiments are still necessary.

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