

Development of natural beverage and a special
components and, methods of mass production and
cultivation technique of *Schisandra nigra* as
special-product of Cheju

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1999. 10. .

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1.

2. RAPD

RAPD (Random Amplified Polymorphic DNA)는 유전자 마커 (genetic marker)로서, DNA의 yield가 PCR (Polymerase Chain Reaction)을 통해 genomic DNA로부터 PCR template DNA, Mg^{2+} , *Taq* DNA polymerase, dNTP, annealing cycle, band pattern 등을 거쳐 RAPD Callus를 생성한다.

가 가

, marker marker
, marker cloning kit 가

3.

,
,
, citral
, total phenol
phenolic acid , ()
, lignan schizandrin,

4.

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,
,
(,)

A.

1.

, 가 가
600 1,350m ,
가 35.
, , 2
, 400m² 3 39 . 3,000 3,094 mm
, 8.8 10.5 0.6
1,300m -7 가
가 50 .
5 6 .
가 , 1:6.3 .
19.2 19.8 . 106.2 ,
9.3 11.1 가 . 0.99g, 722 795
IBA 500ppm
80%가 , 63 92%
WPM BAP GA₃ 1.0
mg/ , BAP 3.0mg/ 가 3

가 200

, 가

가 ,

2. RAPD

genomic DNA

Roggers Bendish (1988), Dellaporta (1983)

genomic DNA ,

DNA .

가 .

가 . RAPD southern hybridization

marker OPA - 17 primer 770 bp, OPB - 03 primer

749 bp, OPB - 9 609 bp,

OPF - 16 1,100 bp, 51 OPA - 17 580 bp

. marker ,

primer . primer , ,

genetic marker .

marker ,

3.

sucrose 1.13% 0.02% ,
fructose glucose 3.71%, 2.51% 1.83%, 1.05%
malic acid 47,684 ppm, 38,691 ppm 가
citric acid 11,939 ppm, 3,330 ppm .
malonic acid maleic acid 2.8 ppm, 2.3 ppm 0.3
ppm, trace .
722 mg% 7,577
mg% , 202 mg%, 5,596 mg%
lysine 300 mg% 288
mg% .
glutamic acid, aspartic acid, threonine, valine Leucine .
1.275%, 1.560%
가
chlorogenic acid 1,276 ppm, 802 ppm , gentisic acid
69 ppm, 136 ppm
ferulic acid 187 ppm, 23 ppm
cinnamic acid, caffeic acid, coumalic
acid .
145 mg/g 160 mg/g

linoleic acid가 21,765 ppm, 45,584 ppm 가 ,
 palmitic acid가 3,974 ppm, 10,872 ppm 가
 . capric acid .
 ,
 ,
 -ylangene, , -elemene, -himachalene,
 -selinene widdrene ,
 caryophyllene, calarene, cubebene, acoradiene -himachalene
 가 .
 86.54mg% , 138.75mg%
 2 .
 peonidin-3-monoglucoside peonidin-3,5-diglucoside ,
 delphinidin-3-monoglucoside malvidin-3- monoglucoside
 .
 schizandrin 0.11%/100g() ,
 0.13%/100g() .

4.

가
 , ,
 가
 가 ()
 (,) 가 가 가
 , , , 가 가
 가 . 80 4 60 6

가 5 6 ,

가 pH

4

7

21

20 (5)

(isoascorbic acid)

20

가

가

B.

가

가

RAPD

genetic marker

marker

가

가

가

가

가

< >

7

RAPD

marker (25(4) 1998.12.)
(12(1) 1998.12.)
(12(1) 1998.12.)
(1999. 6.)

Sex specific marker of *Schisandra nigra* (1999. 10.)

. (1999.12.)
. (2000. 3.)

3 .

(*Schisandra nigra* Max.) ,

(1998. 2.)

DNA marker (2000, 8.)

가 (1999. 2.)

SUMMARY

1. *Schisandra nigra* is a special use species used as a medical and edible fruit. In addition, it is an endemic species which has a unique habitat at the altitude of 600–1,400m in Cheju island. At present, *Schisandra nigra* is confronted with a crisis due to a sudden increase of demand and imprudent destruction of a rare species. Thus, this study was conducted to find 1) multiplication method and genetic variation, 2) ecophysiological characteristics of the natural habitat of *Schisandra nigra* and 3) detection and preservation of genetic resources and upbringing for a superior description.

1.1. *S. nigra* scatteringly grow at 750–1,200m in the western part, at 600–1,100m in the northern part and at 650–1,400m in the southern part. The geographical distribution of *S. nigra* is the secondary forest of broad leaved trees that are mainly composed of *Pinus densiflora*, *Quercus serrata*, *Styrax japonica*, *Lindera erythrocarpa* and *Carpinus laxiflora*. The density of a main distribution sector of *S. nigra* appears in the range of 3–39 individuals in each 20 × 20m plot.

1.2. It was investigated that morphological characteristics on xylem fibers, vessel elements, stomata, and a leaf character of *S. nigra*, *S. chinensis*, *Kadsura japonica*. The results of morphological characteristics were significant at 5% levels within the species and within the individuals as a male, a female and monocious.

1.3. Blooming and fruition of the natural habitat of *S. nigra* were significant

by a region. The most superior region was Youngsil in the western part and Kwaneumsa in the northern part. However, Sanghyo in the southern part was lower than the other regions in the survival rates of a female flower and a quantity of a attached fruit. Fruit characteristics of selected *S. nigra* individuals were averaging at 1.1cm in length, and 741 per liter and 10.3 per cluster in the number of fruits.

1.4. In seed production, the seed germination of the cold moist stratification treatment and the storage in the open ground treatment was the highest in the secondary year(over 93%). In the seed storage and storage pretreatment, the seed current-year germination rate was the highest in the treatment stored at the cold and moist place for 4 mon after immersed in GA₃ 1,000ppm solution during 12 hours.

In cutting reproduction, IBA 500ppm treatment(rooting rate over 80%) showed the higher rate than the other treatments, and the rooting rate by the cutting period was the highest in July(juvenile cutting).

In a grafting reproduction of selected individuals, it indicated a slight difference among individuals, but percents of the graft union was averaging at over 83%.

To propagate the selected individuals by tissue culture, the winter buds were used as a material. Because they were grown in the field, the materials were contaminated after the culture in spite of the surface sterilization. Bus from the two-years-old twigs showed good growth. WPM medium was better than MS and GA₃ was very effective to differentiate shoots from the buds.

1.5. The distribution and frequency of allele to 4 isozymes among 3 populations of *Schisandra nigra* natural habitat showed a polymorphism in *Idh*, *Mdh-2*, *Mnr*, and *Pgi-2* among 6 loci, except *Mdh-1*, *Pgi-1*. All allele(*Idh*, *Mdh-2*, *Pgi-1*, *Mdh-1*, and *Mnr*) except *Pgi-2*, also showed the highest main allele.

2. RAPD (Random Amplified Polymorphic DNA) analysis and southern hybridization were conducted to *S. nigra* plants in order to select the specific markers for monoecious and dioecious individuals. RAPD results using one hundred eighty random 10-mer primers revealed that *S. nigra* had a different banding pattern from *S. chinensis* and *Kadsura japonica*. When DNA isolated from leaves of monoecious and dioecious plants were used as PCR template, only six primers, OPA-17, OPA-19, OPB-03, OPB-09, OPB-16 and OPF-16, showed polymorphic band patterns. No variation in banding profiles within male or female individuals was observed when these six primers were used whereas three monoecious plants(No 6, No 41, No 51) showed different banding patterns one another.

2.1 One primer, OPA-17, yielded DNA fragment of 770 bp, which was detected in male plants but not in any of the female plants tested. By contrast, a 749 bp DNA fragment was amplified in female plants by primer OPB-03, which did not amplified with male DNA samples. We also found three DNA fragments, 609 bp, 1115 bp and 580 bp, which were detected in monoecious but not in male and female plants with primers OPB-09, OPF-16, OPB-03, respectively.

2.2. To verify the specificity of isolated RAPD markers, southern blot hybridization was performed with male, female and monoecious plants DNA. Potential sex-specific RAPD products were isolated from agarose gel and cloned into the pGEM-T Easy vector. Cloned fragments were digested with *EcoRI* and used as probes in southern hybridization analysis of genomic DNA.

2.3. DNAs isolated from male and female plants were separately digested with *EcoRI*, *BamHI* and *HindIII*. When the male and female DNAs were allowed to hybridize with these probes, the 770 bp male probe yielded some bands specific to male plants whereas the 749 bp female probe resulted in single band only for DNA samples from female plants. The 609 bp of monoecious probe hybridized specifically with DNA from three monoecious plants (6, 41, 51), whereas 1,115 bp probe hybridized specifically with DNA from two monoecious plants (41, 51). The 530 bp of monoecious probe hybridized specifically with only one monoecious plant DNA (51). Three monoecious-specific probes did not hybridized with male and female DNA.

2.4. Five RAPD markers found to be sex-specific were sequenced and the homology searches of the data banks were carried out. The sequences did not include long open reading frames and they exhibited no significant similarity to previous reported sequences. Five set of PCR primers were synthesized based on the nucleotide sequences of the cloned fragment, named male 1, female 1, mono 2-1, mono 2-2 and mono 3 and used for the identification of dioecious and monoecious plants by PCR analysis. These primer sets

amplified 460 bp, 436 bp, 411 bp, 684 bp and 207 bp of DNA fragments, respectively. With the designated male and female primer sets, we could identify the sex of dioecious plants. The primer mono 2-1 amplified expected size for all three monoecious plants, whereas mono 2-2 gave a PCR product with two monoecious plants, No. 41 and 51. However, mono 3 primer produced expected 207 bp fragment with only one monoecious plant of No. 51. All monoecious specific primers did not amplify any bands for male or female plants.

2.5. With these primer sets we could easily and rapidly distinguish male, female or monoecious plant within the *S. nigra* population. These results indicate that the five primer sets could be used as genetic markers for the early discrimination of dioecious and monoecious individuals of *S. nigra*.

3. The results from the determination of content of components and biological active substances in *S. chinensis* and *S. nigra* are as follows.

3.1. The contents of free sugar in *S. chinensis* and *S. nigra* was examined. In the *S. chinensis*, content of sucrose was about 1.13%, and there only a few amount of glucose and fructose. But in the *S. nigra*, content of fructose and glucose was 3.71% and 2.51%, respectively, and only a few amount of sucrose.

3.2. In case of organic acid, *S. chinensis* and *S. nigra* contained the higher percentage of malic acid and citric acid than the others. The contents of

malic acid and citric acid in *S. chinensis* was 47,684 and 11,939 ppm/dry weight 100g, and in *S. nigra* was 38,691 and 3,330 ppm/dry weight 100g, respectively.

3.3. Contents of amino acid in *S. chinensis* was higher than that of *S. nigra*. But, content of lysine and arginine in *S. nigra* was higher than that of *S. chinensis*.

3.4. The contents of total phenolic compounds in *S. chinensis* and *S. nigra* was 1.275% and 1.560%, respectively. The predominating phenolic acid was cinnamic acid, gentisic acid, coumalic acid, chlorogenic acid and ferulic acid.

3.5. Contents of crude lipids in *S. chinensis* and *S. nigra*. was 145.5mg/g and 160.5mg/g, respectively. Most of fatty acid in lipids was oleic acid, linoleic acid and linolenic acid as a unsaturated fatty acid, and palmitic acid as a saturated fatty acid.

3.6. In case of essential oils, The predominating components in *S. chinensis* was -ylangene, , -elemene, -himachalene, -selinene and widdrene, *S. nigra* was caryophyllene, calarene, cubebene, acoradiene and -himachalene.

3.7. Contents of total anthocyan pigment in *S. chinensis* and *S. nigra* was 86.54 and 138.75mg%, respectively. Antocyanin, as a aglycone of anthocyan, was supposed to peonidin-3-glycoside in *S. chinensis* and supposed to malvidin-3-glycoside, petunidin-3-glycoside, delphinidin-3-glycoside in *S. nigra*.

3.8. Schizandrin, as a biological active substance, was detected in the extract of *S. chinensis*. Schizandrin-like substance was detected in the extract of *S. nigra*.

4.1. The rate of extract yield from *S. nigra* was best when extracted for 4 hrs. at 80 °C. And we found the possibility that extract from dried stems of *S. nigra* could be used as material for beverage.

4.2. Among Hunter values, both a and b value of the extract from *S. nigra* was much higher than that of *S. chinensis*. As red color was deeper, the extract from *S. nigra* was found to be good in desired process of material to manufacture deep-colored beverage.

4.3. L value, one of Hunter value, and absorbance at 520 nm of the extract from *S. nigra* was low for fresh material, but high for dried material.

4.4. The pH of the extract from *S. nigra* was 0.1-0.2 higher than that of *S. chinensis*. Although the pH of the extract from *S. nigra* was somewhat low when extracted by water, the pH range was to be able to maintain the stability of color the range like *S. chinensis*.

4.5. The sugar content of the extract of *S. nigra* was lower than that of *S. chinensis*, but the difference was insignificant.

4.6. Hunter value a and b of the extract of *S. nigra* extracted for 6hrs. at 60 °C by water was six times higher and 1.5 times higher than that extracted for 24hrs. at room temperature by water. The color of the extract did not

vary when stored in cold for 7 weeks. Hunter value L and absorbance at 520nm was measured by storing at low temperature to investigate the lightness of the extract. Until 4 weeks we found the turbidity increased as L value decreased and absorbance increased. But it seemed to be temporary and little worth consideration because L value and absorbance increased after 5 weeks.

4.7. Total polyphenol of the extract of *S. nigra* decreased during storing period for all samples. Therefore, long-term storage could be the primary factors for browning and altered taste.

4.8. pH of the extract of *S. nigra* decreased but acidity increased during storing period. Therefore, the taste and red color did not change, keeping stability.

4.9. Sugar content of the extract of *S. nigra* did not change at all samples during storing period. For this reason, the quality did not deteriorate easily during storage.

4.10. Beverage products extracted by water, 3% ethanol and aqueous solution from *S. nigra* were stored in cold for 14 weeks and 21 weeks, respectively. Red color was the best for 3% ethanol aqueous solution product at 80 °C, the decrease of which was insignificant during storage. Red color of the extract from dried *S. chinensis* was 1/4 - 1/5 as much as *S. nigra*, which decreased significantly during storage. When we investigated the stability of red color by adding erythrobic acid as antioxidant, it seemed to be effective in spite of a small quantity.

4.11. Lightness of the extract of *S. nigra* was higher than that of *S. chinensis*. Lightness was the highest in the extract of dried *S. chinensis*. These results were considered not to be due to turbidity difference but to inverse proportion to the amount of red color. We confirmed the results by measuring absorbance at 520 nm.

4.12. The content of total polyphenol as a barometer of oxidized browning was measured during storage. Total polyphenol content of *S. nigra* and *S. chinensis* decreased a little until 14 weeks, but much after 21 weeks. On the contrary, total polyphenol content of dried *S. chinensis* decreased very much after 14 weeks, which indicated the possibility to be browned. Total polyphenol content of product added by antioxidant decreased a little until 20 weeks.

4.13. There was no difference in sugar content between *S. nigra* and *S. chinensis*. As sugar content of dried *S. chinensis* was less than half that of *S. nigra*, it seemed that dried *S. chinensis* was not proper to raw material for beverage. Sugar content of extracted product increased during storage, which was considered to be due to altered composition of sugar.

4.14. The acidity of product, which has effects on sour and pH, was the highest for dried *S. chinensis*, in order of *S. chinensis* and *S. nigra*. The acidity of *S. nigra* was 1/3 - 1/4 as much as *S. chinensis*. The acidity decreased a little during storage. Antioxidant had no effect on acidity.

4.15. pH of the extract product of *S. nigra* ranged from 3.0 to 3.5, and that of *S. chinensis* and dried *S. chinensis* ranged from 2.5 to 2.9. pH had no effect on commercial quality of the product. Adding antioxidant was effective to keep pH from decrease.

4.16. The quality of product, adjusting similar red color and having 6% of sugar content by adding sugar, was investigated. Red color of *S. nigra* product was 6 times higher than that of dried *S. chinensis*, thus less sour and fairly sweet.

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4		240
6		241

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genetic marker

RAPD

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(Xij)

vegetational data matrix

$$X_{ij} = (d_{ij} + D_{ij}) / 2 \quad (1)$$

Xij : j i

dij :

Dij :

vegetational data matrix 가 cluster

Ludwig Reynolds가 statistical ecology basic

program . percent dissimilarity (PD)

cluster .

Curtis Mckintosh

(importance value, IV) (2)

(mean importance value, MIV) (6)

$$IV = (RD + RC + RF) / 3 \quad (2)$$

IV :
RD :
RC :
RF :

$$RD = \frac{\text{---}}{\text{---}} \times 100(\%) \quad (3)$$

$$RC = \frac{\text{---}}{\text{---}} \times 100(\%) \quad (4)$$

$$RF = \frac{\text{---}}{\text{---}} \times 100(\%) \quad (5)$$

$$(MIV) = ((IV \times 3) + (IV \times 2) + (IV \times 1)) / 6 \quad (6)$$

Shannon (H')

가

(Maximum H')

$$H'_{max} = \log S(S)$$

$$(J') \quad J' = H' / H'_{max}$$

$$1 - J'$$

$$H' = - \sum p_i \log p_i \quad (7)$$

(, p_i

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Brower Zar

Morista's index

$$\text{Morista index} = n \frac{X^2 - N}{N(N-1)} \quad (8)$$

(, n , N , X)

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Ludwig Reynolds

Pearson Spearman

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F.A.A. (Formalin 5 : Acetic acid

5 : 70% Ethyl alchhol 90) 2 3

Acete-carmin 10 70% ethyl

alcohol glycerin

3)

10%

2 3cm

Schurze 5 7
 safranin 10 70% ethyl alcohol glycerin
 micrometer

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(2)

500mg 가 .
 가 extraction buffer 15,000

rpm 5 , . Gel
 buffer tray buffer Conkle Adams , gel
 12% starch(Sigma) . Buffer starch
 starch gel mold
 gel .
 gel 0.5cm
 gel gel
 gel

1.1. Gel tray Buffer

System	Gel Buffer	Tray Buffer
A Formulation	Tris citrate(pH 8.3) Trizma base ----- 62.0g Citric acid ----- 14.6g Distilled water ----- 10.0 Dissolve the chemicals and check the pH. Store at room temperature	Lithium borate(pH 8.3) Lithium hydroxide---- 12.0g Boric acid----- 118.9g Distilled water----- 10.0 Dissolve the chemicals and check the pH. Store at room temperature
Procedure	To use, add 75ml of the lithium borate buffer to 765ml of the tris citrate gel buffer to make the required 750ml	Use as it is
C Formulation	Tris citrate(pH 6.2) Trizma base 162g Citric acid 108.9g Distilled water 3.0 Dissolve the chemicals and titrate to pH 6.2 with 4N NaOH. Store in the refrigerator	Same as gel buffer Same as buffer
Procedure	To use, mix 16ml of the buffer with 734ml of distilled water.	Mix 250ml of the buffer with 750ml distilled water.

1.2.

System	Stain buffer	Tray buffer system used	Stain components
IDH (Isocitric dehydrogenase)	75ml 0.2M Tris HCl	C	DL- Isocitric acid 200mg NADP 10mg MTT 5mg 1% MgCl ₂ Solution 1mg
MDH (Malic dehydrogenase)	75ml 0.05M Tris HCl pH 8.0	C	Malic acid solution 5ml (134.1g DL-malic acid, 80g NaOH, adjust to pH 7.0 with about nase of 4N NaOH NAD 1ml NBT 1ml PMS 0.5ml
MNR (Menadione reductase)	75ml 0.05M Tris HCl pH 7.0	A	NADH 25mg Menadione 20mg NBT 1mg
PGI (Phosphoglucose isomerase)	75ml 0.05M Tris HCl pH 8.0	A	D- fructose-6 phosphate 25mg 1% MgCl ₂ Solution 1ml NADP 1ml NBT 1ml PMS 0.5ml G6PDH 20 units

aconitase (ACO, E.C. 4.2.1.3), glutamate dehydrogenase (GDH, E.C.1.4.3), glutamate-oxaloacetate transaminase (GOT, E.C.2.6.1.1), isocitric dehydrogenase (IDH, E.C. 1.1.1.42), leucine aminopeptidase (LAP, E.C.3.4.11.1), menadion reductase (MNR, E.C. 1.6.99.2), malate dehydrogenase (MDH, E.C. 1.1.1.37), 6-phosphogluconate dehydro-genase (6PG, E.C. 1.1.1.44), phosphoglucose isomerase (PGI, E.C.5.3.1.9),

phosphoglucomutase(PGM, E.C. 2.7.5.1), shikimate -dehydrogenase(SKDH, E.C. 1.1.1.25) 11 가 GDH, GOT , ACO, LAP, 6PG, PGM 가

IDH, MDH, MNR, PGI 4 , 6 (*Idh, Mdh-1, Mdh-2, Mnr, Pgi-1, Pgi-2*) 가 ,

(*A/L*), (*P*), (*H_o*) (*H_e*) , Hardy - Weinberg Levene X^2 -test cell size가 5 가 Fisher exact-test , X^2 -test exact-test Hardy- Weinberg

Wright *F* , Nei UPGMA PC BIOSYS - 1 program

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H₂O₂ 10%, GA₃ 1,000ppm, BAP 1,000ppm,
 Tween 30%, alcohol 30% 5 , 12
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 4가 4 (120)
 (4) (25) 7
 150 (50 × 3),
 60

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900 m
 IBA ,
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, 1:1:1(v/v) , IBA 500 mg/

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VPER(vermiculite + perlite), VPEA(vermiculite + peatmoss), VPPL(vermiculite+peatmoss+perlite) 1:1:1(v/v) IBA 500mg/

(4)

1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 13, 15, 16, 20 14 7 12 1:1:1(v/v) , IBA 500mg/

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6 41 , 8 , 14 , 22 27 가 가 11 Tween 20 2%

NaClO 20 , 0.2% HgCl₂ 10
4 95% EtOH
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BAP TDZ , BAP GA₃

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	(m)	(.)			
1	840 860	5 10	SW		
2	870 890	5 10	SW		
1	770 790	5 20	E		
1	880 910	10 30	NE NW		
1	780 800	5 30	SE		

600 1,350m ,

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pH 4.36 5.28 , (C.E.C.)

$16.7 \pm 3.83(10.21 \ 22.74)$ me/ 100mg 11.34

me/ 100mg .

1.4.

	(%)	(%)	pH	(me/ 100g)					P ₂ O ₅
				K ⁺	Na ⁺	Ca ⁺⁺	Mg ⁺⁺	C.E.C	
1	22.51	0.61	4.93	0.20	0.16	0.16	0.34	15.74	3.05
2	23.58	1.00	4.37	0.38	0.24	0.40	0.90	12.15	1.30
3	21.27	0.85	5.14	0.26	0.19	0.40	0.50	12.57	0.30
4	25.08	1.33	4.97	0.46	0.33	2.30	1.50	10.21	1.80
5	27.21	0.69	4.92	0.16	0.24	0.30	0.30	13.32	2.02
6	24.86	0.53	5.02	0.35	0.25	2.33	1.48	17.61	2.46
7	24.18	0.96	4.79	0.47	1.43	6.14	0.31	22.19	4.07
8	23.82	0.99	5.28	0.31	0.83	1.40	0.80	16.07	0.30
9	24.51	0.92	4.81	0.42	1.23	3.25	0.56	19.82	3.12
10	22.48	0.61	4.53	0.38	0.33	2.52	0.85	18.22	2.07
11	23.51	1.27	4.90	0.44	0.98	1.70	1.00	19.21	2.40
12	19.27	1.14	4.90	0.35	1.32	0.80	0.60	20.23	1.70
13	15.45	0.68	5.04	0.38	1.43	7.78	3.68	22.74	2.21
14	21.83	0.81	4.58	0.37	0.19	1.00	0.88	16.74	1.88

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2 722 727 (1.6).

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	()	(m)	(cm)	()	()	(g)	(cm)	()
1	50	770- 890	2.32	70.2	10.0	0.83	1.12	740
2	27	850- 890	2.13	64.7	10.4	0.82	1.12	735
2	6	850- 870	2.55	70.3	9.8	0.75	1.09	795
	5	850- 860	2.76	-	-	-	-	-
1	5	770- 790	2.34	63.8	11.1	0.99	1.19	722
2	5	770- 780	2.70	106.2	9.3	0.84	1.11	727
	2	850- 870	2.75	-	-	-	-	-

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A : B : C:



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A : , B : , C :

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	(m)	(cm)	()	()		
					(g)	(cm)
1- 1	880	2.6	16	11.2	0.62	1.03
“ 2	890	1.8	15	11.2	0.65	1.02
“ 3	890	3.0	23	9.2	0.78	1.10
“ 4	880	1.5	20	10.2	0.74	1.10
“ 13	890	2.9	63	9.0	0.53	0.92
2- 5	850	1.5	102	11.8	0.69	0.98
“ 6	850	2.5	135	18.6	0.86	1.10
“ 7	850	1.2	4	10.0	0.60	1.03
“ 8	850	2.5	66	9.6	0.84	1.14
“ 9	850	1.2	11	8.8	0.56	0.94
“ 10	850	2.6	6	10.0	0.59	0.95
“ 11	850	2.0	28	12.0	0.62	0.97
“ 12	850	1.5	9	8.8	0.67	1.01
“ 14	860	1.6	4	8.0	0.72	1.11
“ 15	850	1.5	5	9.6	0.56	0.99
“ 16	850	1.5	16	9.6	0.54	0.99
“ 17	850	2.0	15	11.8	0.61	1.01
“ 18	850	2.0	80	14.2	0.62	1.03
“ 19	850	2.2	8	9.0	0.73	1.11
“ 20	860	1.5	52	8.4	0.49	0.94
“ 21	850	1.9	16	11.4	0.67	1.08
“ 22	850	2.1	6	11.2	0.57	0.99
“ 23	850	1.7	28	11.4	0.65	1.04
“ 24	850	1.8	10	12.6	0.74	1.06
“ 25	850	1.5	47	9.4	0.57	0.99
“ 26	850	2.3	16	12.8	0.60	0.93
“ 27	860	1.3	5	8.8	0.50	0.83
28	790	2.4	62	12.0	0.67	1.00
“ 29	790	2.3	17	10.8	0.71	1.00
“ 30	780	1.8	18	8.6	0.66	1.07

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(m)				
600	5.11 ± 0.70	3.34 ± 0.48	1.91 ± 0.56	0.12 ± 0.02
900	5.16 ± 1.01	3.23 ± 0.60	1.51 ± 0.53	0.12 ± 0.02
1100	5.23 ± 0.71	3.37 ± 0.53	1.81 ± 0.45	0.12 ± 0.01
1300	5.30 ± 0.85	3.29 ± 0.62	1.78 ± 0.54	0.11 ± 0.01
Total	5.20 ± 0.83	3.31 ± 0.55	1.75 ± 0.54	0.12 ± 0.01

600, 900, 1,100, 1,300 m ,

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900 m

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19.

	5.17 ± 0.76	3.31 ± 0.53	1.82 ± 0.36	0.12 ± 0.01
	5.18 ± 0.76	3.30 ± 0.52	1.78 ± 0.44	0.11 ± 0.01
	5.20 ± 0.87	3.29 ± 0.56	1.66 ± 0.51	0.12 ± 0.01
	5.18 ± 0.80	3.34 ± 0.54	1.75 ± 0.45	0.11 ± 0.01

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2)

Perigenous

가 $79.90 \mu\text{m}$

가

$75.20 \mu\text{m}$

92.50 $110.75 \mu\text{m}$

가

가 $694.38/\text{mm}^2$

가

395.33 ,

204 314

, 4 가 , ,
,
가 .

1.10.

	(μm)	(μm)	/	(mm^2)
<i>S. chinensis</i>	79.90 ± 0.351	44.20 ± 0.264	1.939	39.533 ± 4.048
<i>S. nigra</i>	97.50 ± 0.543	63.50 ± 0.812	1.535	31.400 ± 2.472
<i>S. nigra</i> 41	105.88 ± 0.960	58.75 ± 0.244	1.857	20.435 ± 2.635
<i>S. nigra</i> 6	110.75 ± 1.325	64.25 ± 0.504	1.724	23.115 ± 2.207
<i>S. nigra</i>	92.50 ± 0.598	57.75 ± 0.717	1.602	25.714 ± 2.358
<i>Kadsura japonica</i>	76.20 ± 0.268	42.40 ± 0.504	1.797	69.438 ± 4.527

3)

4 , 가
가
752 μm 가 , 가 820 μm , 가 862 947 μm
가
10% 가 .
가 가 , ,
3
가 가

1.11.

	(μm)	(μm)	(μm)	(μm)
<i>S. chinensis</i>	510.80 ± 8.685	64.84 ± 1.282	75.200 ± 12.092	17.20 ± 0.220
<i>S. nigra</i>	702.67 ± 14.431	64.90 ± 1.474	90.333 ± 18.023	21.97 ± 0.381
<i>S. nigra</i> 41	690.00 ± 13.646	71.92 ± 1.393	86.267 ± 16.671	21.50 ± 0.163
<i>S. nigra</i> 6	732.00 ± 12.629	146.66 ± 3.060	94.700 ± 21.011	22.47 ± 0.334
<i>S. nigra</i>	574.33 ± 11.789	91.13 ± 2.310	68.033 ± 19.907	21.87 ± 0.307
<i>Kadsura japonica</i>	680.00 ± 12.628	76.47 ± 2.261	82.000 ± 17.928	20.60 ± 0.178

3

2)

(1)

가 .
 , 가 , (pollen sac) ,
 , 가 , stigmata가 ,
 stigmata가 가 , 가 가
 (1.3). 가
 , 3 .
 . 3
 가 , 3 가 9 가
 . 6 3 .
 , ,
 () ,

가 15 가 , 15
(1.4).

2 3 ,

10 (1.5).

: 1: 6.3 ,

가

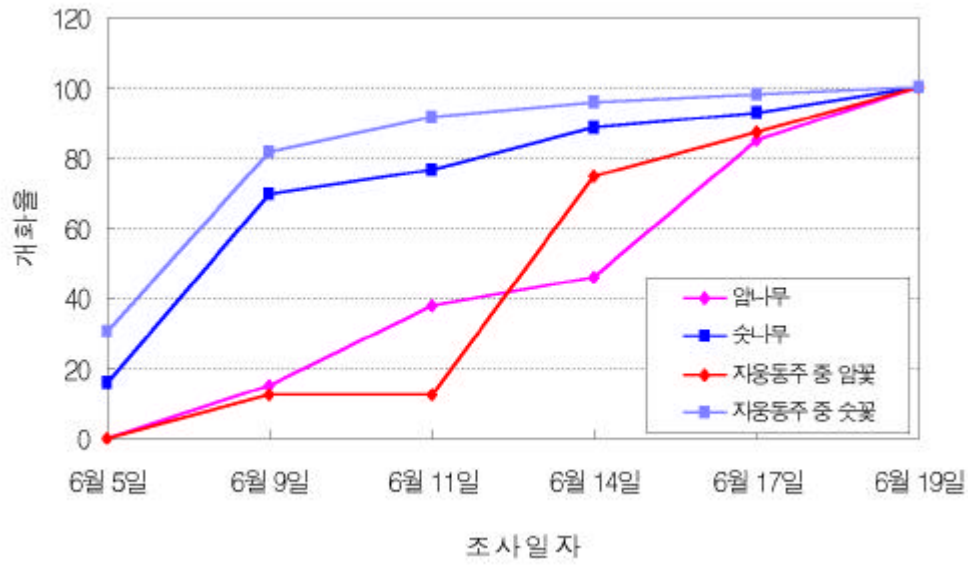
, 가

1.12.

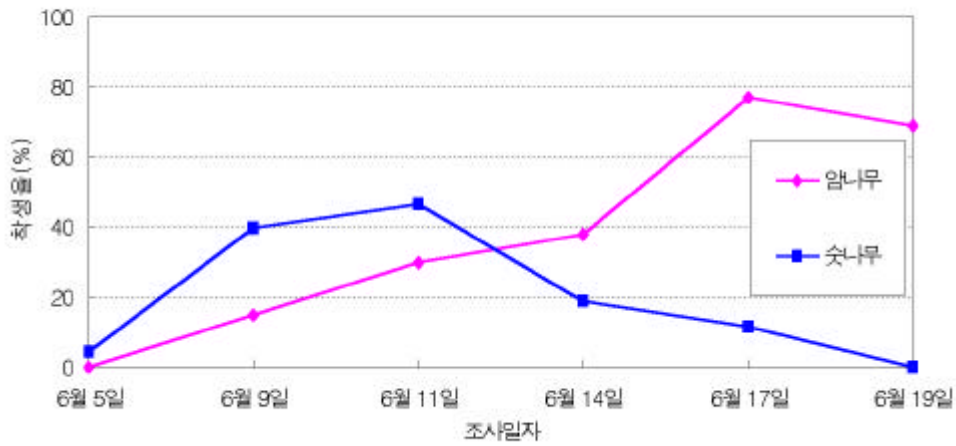
		6 5	6 9	6 14	6 17
(cm)		-	5.35 ± 1.44	5.49 ± 1.52	5.59 ± 1.56
		-	3.00 ± 0.48	3.02 ± 0.49	3.06 ± 0.62
		7.04 ± 0.43	7.08 ± 0.57	7.21 ± 0.64	7.52 ± 0.49
		3.35 ± 0.62	3.50 ± 0.81	3.76 ± 1.75	-



1.3. (A: , B: , C:)



1.4.



1.5.

(2)

5 , ,
21.2 , 14.4
68.0% .

905g (1.13).

1.13.

	(m)	(cm)	()	()	(%)	(g)
1	850	2.5	20.8	12.8	62	710
2	850	2.5	24.5	18.6	76	2,159
3	890	2.9	20.1	14.2	71	744
4	850	2.0	20.8	14.2	68	704
5	850	2.0	19.6	12.0	61	208
		2.4	21.2	14.4	68	905

4 4

, , , 19.22 19.82
가 , 1.8 10.5 가
. 가 10.5 54%
(1.14).

1.14.

	(m)	()	()	(%)
	850-890	19.32	8.9	46
	770-790	19.51	10.5	54
	550-800	19.22	1.8	9
	650-750	19.82	6.5	33
	550-750	19.50	6.9	36

4.

1)

(1)

6 가 $M dh-1$ $P gi-1$
 2 가 4
 (1.15). $P gi-2$ 가
 가 가 , $P gi-2$
 3 가 2
 , b 가
 a 가
 $I dh-1$ a 가 0.033
 , $M dh-2$ 3
 가 , 1 .

Population				Population			
Locus	1	2	3	Locus	1	2	3
<i>Idh</i>			<i>Mdh-1</i>				
(N)	30	30	30	(N)	30	30	30
a	.033	.000	.000	a	1.000	1.000	1.000
b	.967	1.000	1.000				
<i>Mdh-2</i>			<i>Mnr</i>				
(N)	30	30	30	(N)	30	30	30
a	.083	.000	.133	a	.783	.833	.983
b	.883	1.000	.850	b	.217	.167	.017
c	.033	.000	.017				
<i>Pgi-1</i>			<i>Pgi-2</i>				
(N)	30	30	30	(N)	30	30	30
a	1.000	1.000	1.000	a	.250	.283	.617
				b	.683	.667	.383
				c	.067	.050	.000

N: , 1: Youngsil() 2: Sanghyo(); 3: Kwaneumsa()

(2)

1. 26

. 1.7 , 95% 99%
 38.9% 50.0% ,
 0.141 0.147 .
 , 가
 . 가
 , .
 96

($A/L=1.68$; $P=45.1\%$; $H_e=0.143$: Hamrick et al., 1992)

가 (endemic: $A/L=1.48$; $P=26.3\%$; $H_e=0.056$)

($P=67.7\%$, $A/L=2.15$, $H_e=0.251$)

($A/L=1.90$; $P=60.3\%$; $H_e=0.208$)

가

1.16.

Population	A/L	P_{95}	P_{98}	H_o	H_e
Youngsil	2.0 (.4)	50.0	66.7	.172 (.077)	.183 (.080)
Sanghyo	1.5 (.3)	33.3	33.3	.111 (.070)	.127 (.084)
	1.7 (.3)	33.3	50.0	.139 (.087)	.130 (.082)
	Mean	1.7	38.9	50.0	.141

Note: A/L , number of alleles per locus; P_{95} , percentage of polymorphic loci at 95% level; P_{99} , percentage of polymorphic loci at 99% level; H_o , observed heterozygosity; H_e , Nei's unbiased expected heterozygosity

가

가

가

,

가

가

가

,

.

,

가

가

(鳥)

,

.

,

가

,

가

子

가

가

.

가

가

.

(3) Wright F

Wright F F_{IS} F_{IT} 0 가

Hardy - Weinberg

().

Hardy - Weinberg

,

Hardy - Weinberg

가

가

Hardy - Weinberg

가

,

가

가

1.17.

Wright F

Locus	F_{IS}	F_{IT}	F_{ST}
<i>Idh</i>	-.034	-.011	.022
<i>Mdh-2</i>	.009	.054	.045
<i>Mnr</i>	-.231	-.154	.063
<i>Pgi-2</i>	.150	.227	.091
Mean	.025	.097	.074

가 7.4% ($F_{ST}=0.074$),
 92.6%

가 ($G_{ST}=0.124$)
 가 ($G_{ST}=0.141$)
 가
 가

pattern

가

(4) Nei

Nei (D) (I)
 0.018 0.983 F_{ST} 가

1.18.

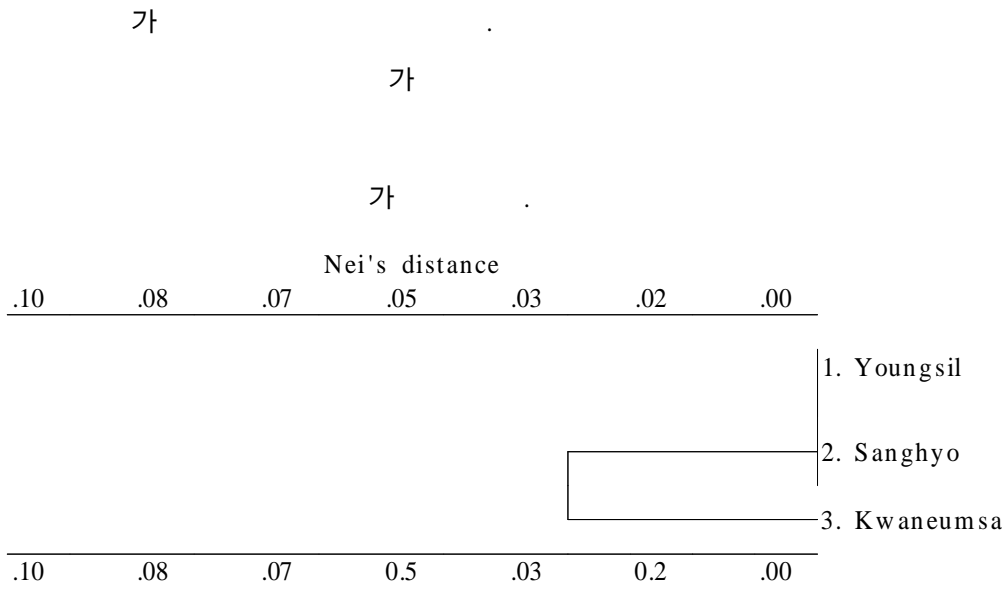
Nei

Population	1	2	3
Youngsil	*****	.000	.028
Sanghyo	1.000	*****	.025
Kwaneum sa	.972	.976	*****

UPGMA

1.6

Pgi-2



Cophenetic correlation = .994

1.6. Nei

5.

1)

가 . 1995 9 20 1995 10 5
 果肉
 4 , 4 , ,
 4 5 300
 2 1.19 .

1.19.

	()		
		(%)	2 (%)
	300	0	61
	300	9	95
	300	8	93
	300	0	38

가

8 9% ,

가 . 2

가 93 95%

38 61%

가

가

, H₂O₂ 10%, GA₃ 1,000ppm, BAP 1,000ppm,

TWEEN Alcohol 30% 12 , ,

25 , 4 , 4 7 , 25 7

5 × 4 , 60

GA₃ 1,000ppm 가

15 29% 가 ,

가 가 . GA₃ 가 가 29%

9% .

가 3 가

Hartman Kester 가

,

가 2 가 , 4가 5
 95% 93%
 (1.20).

1.20.

		()	()	(%)
4	H ₂ O ₂	150	20	13
	GA ₃	150	44	29
	TWEEN	150	28	19
	Alcohol	150	0	0
	BAP	150	0	0
2 + 3	H ₂ O ₂	150	16	4
	GA ₃	150	38	14
	TWEEN	150	32	10
	Alcohol	150	11	4
	BAP	150	5	2
4	H ₂ O ₂	150	16	3
	GA ₃	150	22	7
	TWEEN	150	15	5
	Alcohol	150	7	3
	BAP	150	0	0
4	H ₂ O ₂	150	14	4
	GA ₃	150	26	10
	TWEEN	150	18	6
	Alcohol	150	5	2
	BAP	150	0	0
24		150	33	14
4		150	0	0

2)

(1)

IBA 69%, NAA 55%, IAA 52%,
 52%, IBA가 가 NAA 50 mg/ 가 30% 가 .
 IBA 가 6.6 , 6.1 cm 가 , 가 47 , IAA 가 5.1 cm 가 .

1.21.

(mg/)	()	()	(%)	()	(cm)
IBA 50	60	34	57	5.7	6.4
100	60	46	77	6.6	6.2
500	60	48	80	6.4	6.1
1000	60	37	62	7.6	5.8
Mean	60	41	69	6.6	6.1
NAA 50	60	18	30	4.8	5.2
100	60	38	63	5.4	5.3
500	60	46	77	5.9	6.2
1000	60	29	48	6.1	5.6
Mean	60	33	55	5.6	5.6
IAA 50	60	24	40	4.8	5.4
100	60	29	48	4.6	5.9
500	60	38	63	4.9	4.9
1000	60	34	57	4.3	4.2
Mean	60	31	62	4.7	5.1
	60	31	52	4.7	5.8

7 10 IBA 500 mg/ 80%
 가 , 8 24 가
 48% 가 가 .
 7 10 가 73% 가 8 24
 가 63% 가 8

3

가

7

IBA 500

mg/

가 가

Table 1.22. IBA

	3 20	6 10	8 1	8 24
IBA 50ppm	57	63	68	55
100	77	78	78	77
500	80	83	78	78
1000	62	80	58	57
	52	60	57	48
	66	73	67	63

IBA

500mg/

가

가

(2)

63 88%

가

5.9 9.6 ,

가

5.8 7.3 cm,

6.1 9.0 cm

가

가

, Kim Nam

5, 10, 20

가

85.7%, 81.7%, 62.4%

Yim 1, 2, 10, 20 가 84%, 43%, 28%, 15% 15 63%가

1.23.

	()	()	(%)	()	(cm)	(cm)	(mm)
1	60	53	88	9.6	7.3	9.0	1.2
5	60	49	82	8.1	6.9	7.5	1.3
10	60	45	75	7.3	6.2	7.4	1.2
15	60	38	63	5.9	5.8	6.1	1.2
	60	46	77	7.7	6.6	7.5	1.2
F-			15.90**	12.78**	9.30**	9.42**	0.97 ^{NS}

** : significant at 1% level

NS : Non significant at 1 or 5% level

(3)

50%가 60%, 67%, 가 가 5.0 7.4 , 5.7 6.7cm, 5.7 7.0cm 1.0 2.1mm 가

1. 24.

	()	(%)	()	(cm)	(cm)	(mm)
	60	36	60	7.1	6.4	6.8
	60	40	67	7.4	6.7	7.0
	60	30	50	5.0	5.7	5.7
	60	35	59	6.5	6.3	6.5
F-			4.618**	89.36**	13.80**	27.40**
						6.89**

** : significant at 1% level

NS : Non significant at 1 or 5% level

Kummerow

가 가

Farrar and Grace *Pinus monticola*

가 가

가

가

Table 21

VPPL 가 68%가 가

, 가

38%

가

가

6.0 6.5cm, 6.4 6.7cm 2.0 2.1 mm 6.2 7.0 ,

1.25.

	()	()	(%)	()	(cm)
	60	23	38	6.2	6.0
VPER	60	38	63	6.6	6.5
VPEA	60	39	65	6.3	6.2
VPPL	60	41	68	7.0	6.3
	60	35	59	6.5	6.3
F-			17.31**	0.868 ^{NS}	0.826 ^{NS}

*VPER:vermiculite+perlite 1:1(v/v), VPEA:vermiculite+peatmoss 1:1 (v/v), VPPL:vermiculite+peatmoss+perlite 1:1(v/v), ** : significant at 1% level, NS : Non significant at 1 or 5% level

가

가

가

(4)

40 85% .
 2, 9, 25 가 80% 가 가 , 10
 가 40% 가 . 5.0 7.6 ,
 5.4 7.4cm .

가

Table 1.26.

	()	()	(%)	()	(cm)
1	20	15	75	6.4	7.4
2	20	17	85	5.8	6.8
3	20	11	55	6.2	6.9
4	20	13	65	7.6	6.6
5	20	16	80	5.2	5.4
6	20	9	45	6.4	6.6
7	20	12	60	5.2	6.3
8	20	14	70	5.0	5.8
9	20	17	85	7.3	6.8
10	20	8	40	6.6	6.4
13	20	13	65	6.2	7.6
15	20	17	85	5.8	7.8
16	20	15	75	6.2	6.4
20	20	10	50	5.6	6.8
	20	13	67	6.1	6.7

3)

30

63 92%

(1.27).

83%

가

1.27.

	()	()	(%)		()	()	(%)
1	24	20	83	30	24	15	63
2	24	17	71	31	24	18	75
3	24	22	92	32	26	17	65
4	24	21	88	33	18	13	72
5	24	20	83	34	25	19	76
6	24	22	92	35	30	18	60
7	24	21	88	36	20	10	50
8	24	18	75	37	22	12	55
9	24	20	83	38	26	15	58
10	24	18	75	39	24	20	83
12	24	23	72	40	24	18	75
13	24	22	92	41	24	18	75
14	24	21	88	42	22	14	64
15	24	28	75	43	22	17	77
19	24	16	73	46	24	14	70
20	24	20	83	47	26	20	83
22	24	16	73	48	28	11	42
28	24	17	72	49	24	18	64
30	24	15	63	50	26	17	71
					983	702	71

4)

. 6 41 , 8 , 14 , 22 27

(1)

가 11 가
가 .

1.29.

(6)

(mg/)		
WPM 가	4.4 ± 1.0	3.9 ± 1.2
BAP 1.0	2.9 ± 0.9	2.7 ± 1.0
GA ₃ 1.0	3.2 ± 1.5	3.8 ± 1.4
BAP 1.0+GA ₃ 1.0	3.2 ± 0.7	2.9 ± 0.9
MS 가	3.5 ± 1.5	3.8 ± 1.3
BAP 1.0	2.8 ± 1.1	1.9 ± 0.8
GA ₃ 1.0	2.8 ± 1.1	2.7 ± 1.1
BAP 1.0+GA ₃ 1.0	2.7 ± 0.8	2.2 ± 0.8

2 4 ,

가

1.30.

(6)

(mg/)		
WPM 가	76.2 ± 21.1	74.6 ± 22.9
BAP 1.0	100.5 ± 37.6	80.9 ± 26.6
GA ₃ 1.0	80.4 ± 36.0	97.7 ± 20.7
BAP 1.0+GA ₃ 1.0	120.6 ± 57.4	91.9 ± 31.1
MS 가	75.3 ± 38.1	82.2 ± 27.7
BAP 1.0	98.3 ± 39.5	82.0 ± 31.2
GA ₃ 1.0	88.0 ± 32.9	87.1 ± 40.4
BAP 1.0+GA ₃ 1.0	126.0 ± 45.7	82.4 ± 37.4

BAP 1.0mg/ WPM MS BAP GA₃가 , 가

1.31. (%)

(mg/)		
WPM 가	0	0
BAP 1.0	9.1	0
GA ₃ 1.0	55.6	19.4
BAP 1.0+GA ₃ 1.0	23.1	4.0
MS 가	0	0
BAP 1.0	0	0
GA ₃ 1.0	35.7	14.3
BAP 1.0+GA ₃ 1.0	11.8	0

가 가 GA₃가
 GA₃ BAP가 가
 가
 GA₃가 BAP가
 WPM MS WPM

BAP 2.0mg/ TDZ 0.05 0.1mg 3
 가 가 GA₃
 가

1.32.

BAP 0.5	264.8 ± 165.3	1.1 ± 1.1	10.6 ± 5.9
BAP 1.0	243.5 ± 113.3	2.1 ± 1.9	8.0 ± 3.6
BAP 2.0	277.8 ± 154.6	3.0 ± 2.5	7.3 ± 3.0
BAP 3.0	267.6 ± 59.4	2.3 ± 2.3	7.5 ± 3.8
BAP 5.0	315.6 ± 140.9	1.9 ± 1.7	9.4 ± 5.5
TDZ 0.05	349.7 ± 185.2	2.8 ± 2.9	7.4 ± 3.3
TDZ 0.1	302.2 ± 146.1	2.0 ± 1.8	7.9 ± 3.9
TDZ 1.0	296.3 ± 169.4	2.8 ± 2.5	7.4 ± 3.9

BAP GA₃

BAP GA₃

GA₃

가 BAP

GA₃ 3.0mg/

BAP 2.0mg/ 가 가

가

(1.32.).

1.33.

BAP 1.0 + GA ₃ 1.0	218.9 ± 89.1
BAP 1.0 + GA ₃ 2.0	159.5 ± 72.3
BAP 1.0 + GA ₃ 3.0	217.5 ± 74.7
BAP 2.0 + GA ₃ 1.0	194.3 ± 107.5
BAP 2.0 + GA ₃ 2.0	251.8 ± 126.2
BAP 2.0 + GA ₃ 3.0	153.9 ± 77.8
BAP 3.0 + GA ₃ 1.0	169.3 ± 68.6
BAP 3.0 + GA ₃ 2.0	258.3 ± 92.1
BAP 3.0 + GA ₃ 3.0	172.3 ± 123.4

, (1.7).

5)

18m² 1 , 36m² 1 ,
 170m²
 25 86 (1.8).
 1997 1999 4 가 (1.34).
 26.4% , 327 , 248
 , 6.3:1 .

1.34.

()	()	(%)		
125	33	26.4	327	248



17. (A : , B : C :)



18. Pergola

4

, 가 가

600 1,350m

가 35.

2 , 400m²

3 39 3,000 3,094 mm

8.8 10.5 0.6

1,300m -7 가

가 50 5

6 가

, 1:6.3 19.2 19.8

. 106.2 , 9.3

11.1 가 0.99g, 722 795 .

IBA 500ppm 80%가

, 63 92% .

WPM BAP GA₃ 1.0 mg/

, BAP 3.0mg/ 가 3

, 가 200

, 가

가 ,

3 RAPD

1

RAPD ,
genetic marker .
DNA ,
DNA yield 가 PCR ,
genomic DNA .
PCR template DNA, Mg²⁺, Taq DNA polymerase, dNTP ,
annealing cycle band pattern 가 RAPD
가
Callus
가 가
marker marker
marker cloning kit 가

2

1.

Schisandra 19
3 (6 , 41 , 51), 8 (46, 45 , 47 , 48 ,

49 , 50 , 52 , 53), 7 (29 , 32 , 33 , 42 , 44 , 54 , 55)
1 *Kadsura japonica* .

2. Genomic DNA

500 mg
10 가 isolation buffer [10% polyethylene glycol 6000, 0.35 M sorbitol, 0.1 M Tris (pH 8.0), 0.5% spermidine, 0.5% spermine, 0.5% -mercaptoethanol] , (4 , 15,000 rpm, 10)
5 lysis buffer [0.35 M sorbitol, 0.1 M Tris (pH 8.0), 0.5% spermidine, 0.5% spermine, 0.5% -mercapto -ethanol] 1/10 10% sarcosine 가 , 10 , 2 CTAB [2% cetyltrimethylammonium bromide (CTAB), 0.1 M Tris (pH 9.5), 20 mM EDTA, 1.4 M NaCl, 0.5% -mercaptoethanol] , 65 10 . chloroform (chloroform isoamylalchol = 24:1) 가 (25 , 15,000 rpm, 10) , isopropanol . DNA pipet 가 99.9% ethanol tube , 가 70 80% ethanol 1 Mℓ 가 , . 200 μℓ TE buffer [10 mM Tris (pH 8.0), 1 mM EDTA] , phenol:chloroform (1:1) (25 , 15,000 rpm, 10) . isopropanol , 99.9% ethanol 1 Mℓ 가 (4 , 15,000 rpm, 10) . 70% ethanol (4 , 15,000 rpm, 10) . DNA TE buffer [10 mM Tris (pH 8.0), 1 mM EDTA]

EDTA가 PCR buffer Mg²⁺

DNA Hoefer DNA fluorometer (TKO-100)

3. Random primers

PCR Primer Operon Technologies, Inc. random primer OPA kit (20 primers), OPB kit (20 primers), OPC kit (20 primers), OPD kit (20 primers), OPE kit (20 primers), OPF kit (20 primers), OPG kit (20 primers), OPH kit (20 primers), OPI kit (20 primers) 180 primer

4. PCR

가. RAPD (Random Amplified Polymorphic DNA)

PCR 20 ng template DNA, 25 pmole primer (decanucleotide), 200 μM deoxyribonucleic acid, 1.5 mM MgCl₂, 1/10 10 × PCR buffer [100 mM Tris-HCl, 25 mM MgCl₂, 500 mM KCl (pH 8.3)], 1.25 unit *Taq* DNA polymerase (Takara) 25 μl PCR Perkin Elmer GeneAmp PCR system 2400 94 5 denaturation, 94 30 denaturation, 37 30 annealing, 72 30 extension 45 72 5 PCR 1% agarose gel ethidium bromide

marker PCR

PCR 94 5

denaturation, 94 30 denaturation, 55 30
 annealing, 72 30 extension 30 72
 5 . PCR 1.2% agarose gel
 ethidium bromide .

5. Agarose gel DNA

RAPD DNA , marker
 band agarose gel GENE CLEAN kit
 (Bio101) . gel 3 NaI
 , 45 55 5 . 5 $\mu\ell$ glassmilk
 , 5 . (15,000 rpm, 5)
 200 $\mu\ell$ New wash (New concentrate 14 $M\ell$, H₂O 280 $M\ell$, 99.9%
 ethyl alcohol 310 $M\ell$) 3 . TE buffer 4
 5 55 3 (15,000 rpm, 3)
 . DNA sample Hoefler DNA fluorometer (TKO-100)
 .

6. marker cloning

, marker band
 , pGEM-T Easy Vector System (Promega) cloning
 . insert vector 3:1 가 . DNA
 1 $\mu\ell$ T4 DNA ligase 10 \times buffer, pGEM-T easy vector (50 ng, 2-1) 1
 $\mu\ell$, T4 DNA ligase (3 Weiss units/ $\mu\ell$) , 10 $\mu\ell$
 . 4 16 24 .
 ligation 50 $\mu\ell$ competent cell

, 20 . 42 45 50
 , 950 $\mu\ell$ SOC medium [0.5% (w/v)
 yeast extract, 2% (w/v) tryptone, 10 mM NaCl, 2.5 mM KCl, 10 mM MgCl₂,
 20 mM MgSO₄, 20 mM glucose] , 37 1
 30 . ampicillin (50 $\mu\text{g}/\text{M}\ell$), 20 $\mu\ell$ X-gal (50
 mg/M ℓ in dimethylformamide), 100 $\mu\ell$ IPTG (100 mM) 가 LB (tryptone
 10 g, yeast extract 5 g, NaCl 5 g, 1 M NaOH 1 M ℓ /) 37
 16 24 . colony

plasmid DNA *EcoR*
 marker .

7. callus

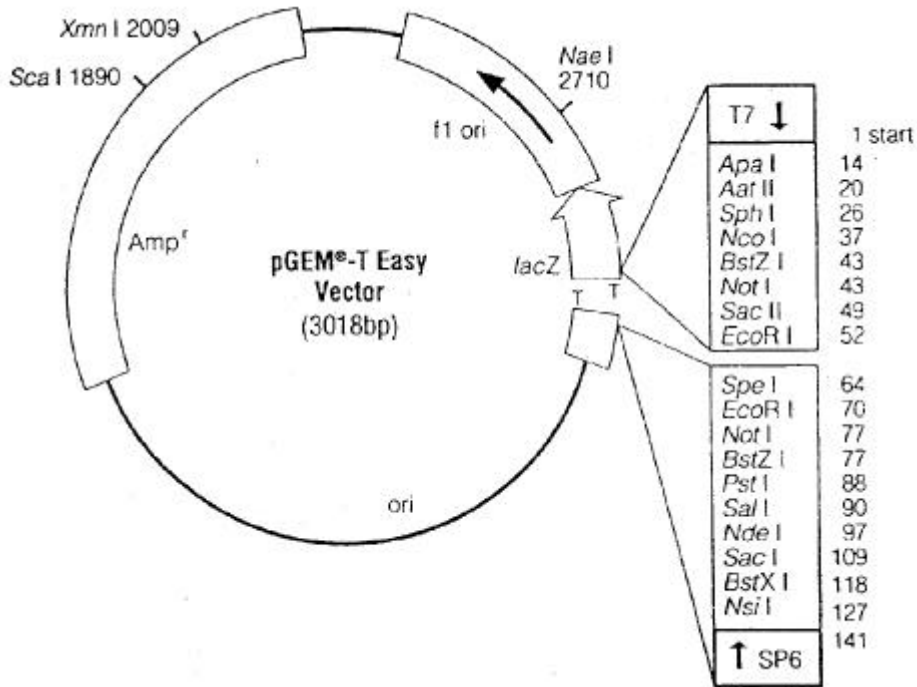
70% 30 2% sodium
 hypochlorite 15
 . (NAA, BA) MS,
 WPM, GD callus .

8. Plasmid DNA

가. Cloning plasmid DNA

Cloning colony ampicillin (50 $\mu\text{g}/\mu\ell$) 가 LB 37
 saturation , WizardTM Minipreps DNA Purification
 System (Promega) plasmid DNA . 1.5 M ℓ
 eppendorff tube 15,000 rpm, 1 cell
 resuspension solution (50 mM Tris-HCl, pH 7.5, 10 mM EDTA, 100 $\mu\text{g}/\text{M}\ell$)
 , 200 $\mu\ell$ cell lysis solution

200 $\mu\ell$ neutralization solution
 15,000 rpm 5 1 M ℓ
 WizardTM Minipreps DNA



2.1. pGEM-T easy vector

pGEM-T vector pGEM-5zf(+) DNA *EcoR* ,
 thymidine 3'-T overhang PCR ligation
 가 purification resin , resin/DNA
 WizardTM minicolumn elution DNA 2 M ℓ
 column wash solution , 4 , 15,000 rpm 2
 30 . 60 20 $\mu\ell$ DNA

4 , 15,000 rpm, 2 DNA .

. Sequencing plasmid

Cloning colony ampicillin (50 $\mu\text{g}/\mu\text{l}$) 가 2 M ℓ LB

37 saturation 500 M ℓ LB

overnight . centrifuge tube 5 ,

4 , 7,000rpm, 5 . 18M ℓ TEG (50

mM glucose, 10 mM EDTA, 25 mM Tris-HCl, pH 8.0) .

(lysozyme 10 mg / TEG M ℓ) 2 M ℓ 가 , 10

. 40 M ℓ (0.2 N NaOH, 1% SDS)

, 10 . 30 M ℓ 3 M potassium acetate (pH

5.5) , 15 4 , 8,000 rpm, 5

. (cheese cloth) tube

60 M ℓ 가 isopropanol 가 , deep freezer (-70) 30

. 4 , 12,000 rpm, 15 10

4.3 M ℓ 가 . 4.8 g

CsCl, 0.5 M ℓ EtBr (10 mg/M ℓ) , 4 . 12,000 rpm

15 . 18 gage

tube , (Beckman) , 20 ,

50,000 rpm, 17 . 21 gage

tube , 18 gage plasmid

DNA tube , 20 SSC isopropanol

EtBr . , TE

buffer [10 mM Tris (pH 8.0), 1 mM EDTA]

DNA . DNA sequencing

9.

Boehringer Mannheim . DNA TE
buffer 37 1 3 . 65
80 10 , 1/6
(50% glycerol, 0.1 M EDTA, 0.025% bromophenol blue, 0.025% xylene cyanol)

10. Southern hybridization

, marker
southern hybridization . probe recombinant plasmid
DNA *EcoR* Gene clean kit agarose gel
. DNA DIG DNA labelling and detection kit
(Boehringer Mannheim Co.) labeling probe .
probe DNA 15 $\mu\ell$ 95 10
3 . 2 $\mu\ell$ hexanucleotide mixture, 2 $\mu\ell$ dNTP labelling
mixture, 1 $\mu\ell$ Klenow enzyme (100 U/ $\mu\ell$) 가 37 60
. 2 $\mu\ell$ 0.2 M EDTA (pH 8.0), 2.5 $\mu\ell$ 4 M LiCl, 75 $\mu\ell$ 가
ethyl alcohol 가 -70 30 .
(4 , 15,000 rpm, 15) 50 $\mu\ell$ 70% ethyl alcohol
DNA . DNA 50 $\mu\ell$
TE buffer probe .
agarose gel gel denaturation solution (1.5
M NaCl, 0.5 N NaOH) 45 . Gel
, neutralization solution [1 M Tris (pH 8.0), 1.5 M NaCl]
45 . Nylon membrane (Boehringer

11. **Primer**

4 μg DNA 18 μl , 2 μl 2M NaOH, 2 mM EDTA
 , 5 . 75 μl 99.9% ethylalcohol
 , -70 10 . 4 , 14,000 rpm 10
 20 μl 가 70% ethylalcohol 1 .
 18 μl . ALF-Express
 automatic sequencer (Pharmacia Biotech) sequencing .
 sequence homology <http://www.ncbi.nlm.nih.gov/BLAST/>
 Basic Local Alignment Search Tool (BLAST) program
 , marker **Primer**
 Primer3 Input (<http://www.genome.wi.mit.edu>) site , primer
 가 , () primer

3

1. genomic DNA

CTAB (cetyltrimethylammonium bromide) spermine-spermidine
genomic DNA 1 g 1.6 mg
genomic DNA . CTAB
(Rogers and Bendish, 1988) Dellaporta genomic DNA
, DNA
genomic DNA 가

2. PCR

RAPD marker PCR 가
annealing , 36 55 가
(Williams , 1990; Hu and Quiros, 1991; Demeke , 1992; Kim , 1997).
, , 90% RAPD marker
annealing 36 (Koller , 1993; Adams , 1993) , 37 (Bellamy
, 1996; Lee , 1995) . denaturation 94 ,
extension 72 , annealing 37 , 42 , 50
PCR , 37 가 band
pattern .
cycle 37 annealing , 30cycle,
35cycle, 40cycle, 45cycle , 45cycle
PCR DNA band pattern

10 ng, 15 ng, 20 ng, 30 ng, 50 ng, 100 ng DNA 가 PCR
 , 10 ng, 15 ng band 가 , 100 ng
 band smear band pattern . (Adams , 1993,
 (Koller , 1993), (Lee , 1995) 30 ng DNA
 band pattern .

PCR annealing 37 , 45cycles , DNA

30 ng/25 μ l , 3

3. ,

primer 10-mer OPA kit (20 primers), OPB kit
 (20 primers), OPC kit (20 primers), OPD kit (20 primers), OPE kit (20
 primers), OPF kit (20 primers), OPG (20 primers), OPH kit (20 primers),
 OPI kit (20 primers) 180 primer .

2 1 RAPD .

2-2 , 180 primer 9가 primer

, 180 primer , , 3

band pattern . OPD-15 primer

800 bp, 2,500 bp, 1,900 bp

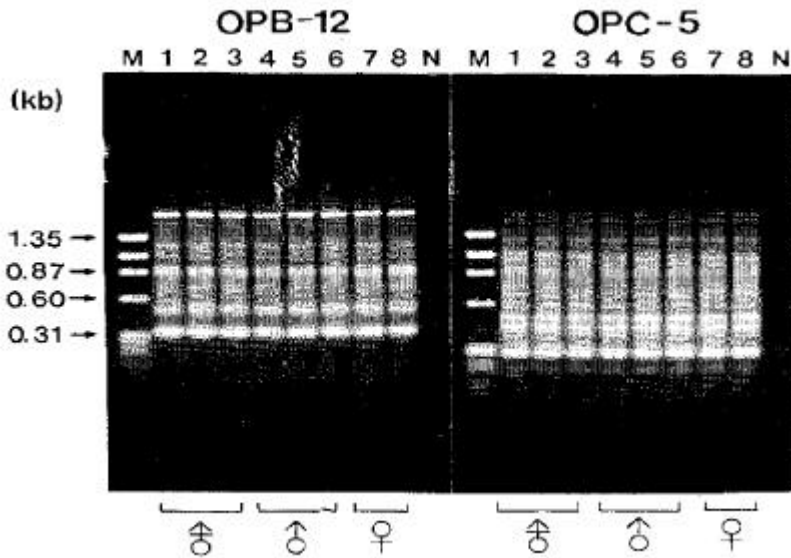
band가 . 9 primer 가

band가 128 , 1 10 band pattern

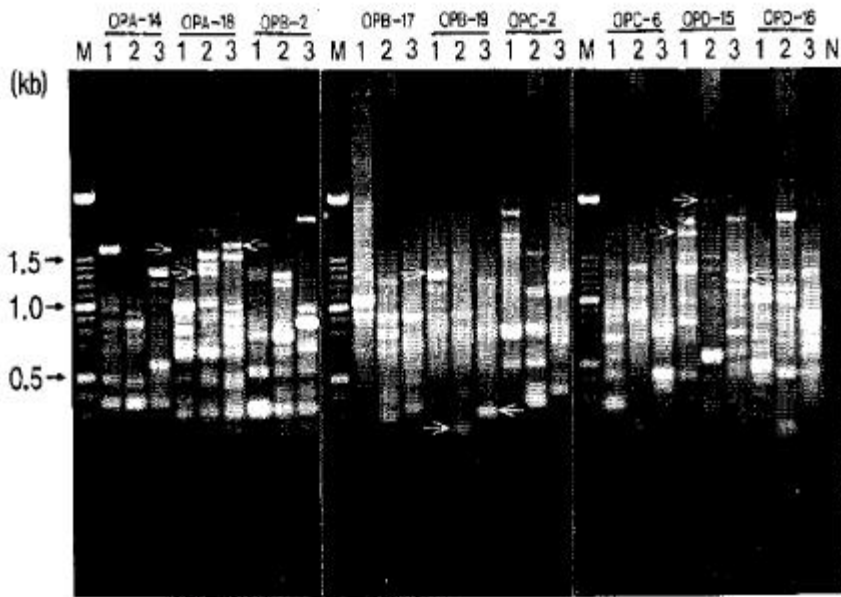
band pattern 150-3,000 bp .

4. marker

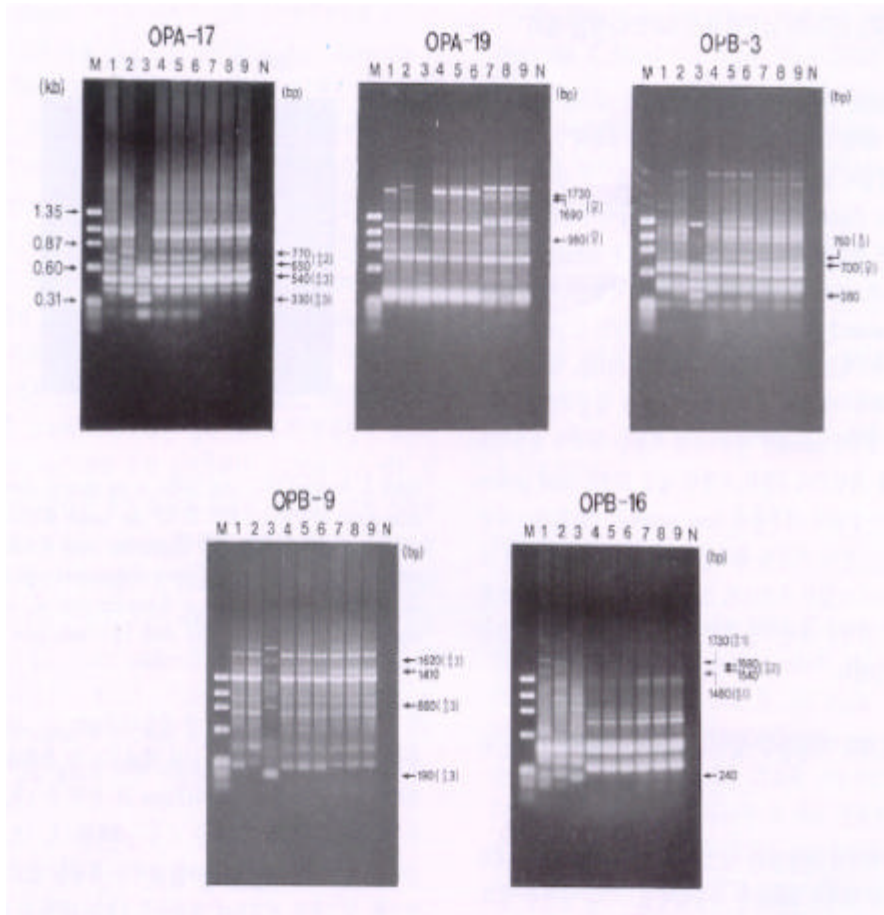
, , 3 180 primer
 RAPD , 174 primer , ,
 primer band pattern (2.3).
 6 random primer (2.1) polymorphic band pattern
 band가 (2.4, 2.5).



2.2. Primer OPB-12 OPC-5 PCR . Lane M: size
 marker, *Hae* X174 DNA; lane 1-3: 6, 41, 51;
 lane 4-6: 46, 52, 53; lane 7-8: 42, 54; N:

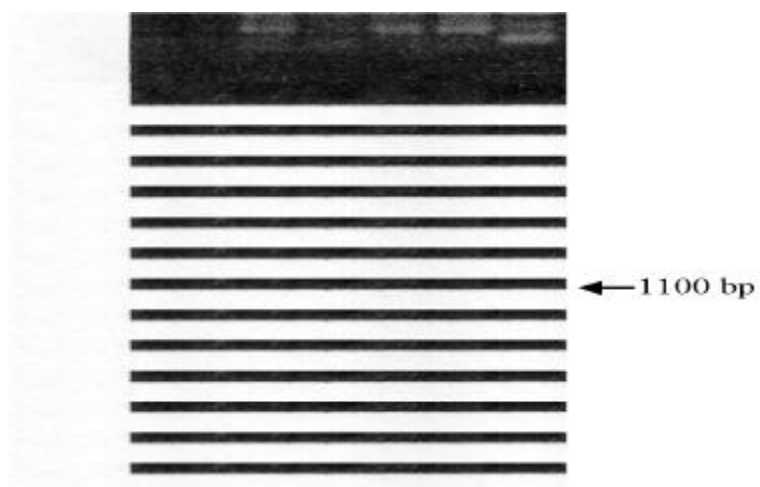


2.3. **Primer** **PCR** . Lane M: size marker 100 bp ladder; lane 1: ; lane 2: ; lane 3: ; lane N: ; band .



2.4. Primer OPA - 17, OPA - 19, OPB - 03, OPB - 9, OPB - 16

PCR . Lane M: *Hae* X174 DNA; lane 1-3: 6, 41, 51; lane 4-6: 46, 52, 53; lane 7-8: 42, 52, 54; lane N:



2.5. OPF-16

PCR

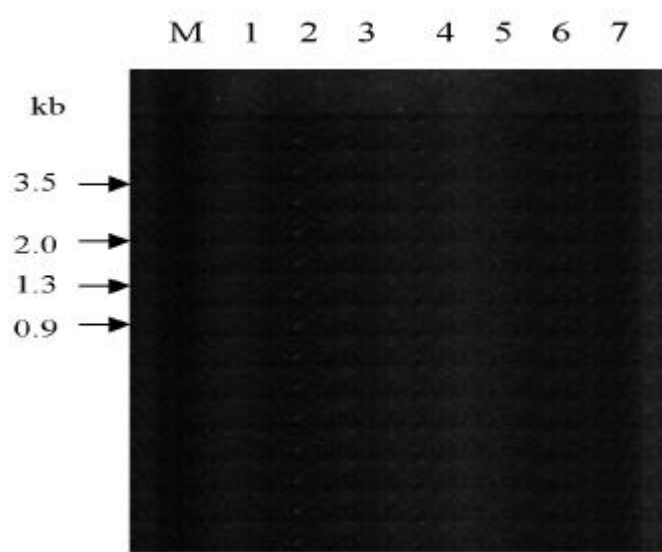
Lane M: DNA *EcoR* *Hind* DNA; lane 1:
 6; lane 2: 41; lane 3: 51; lane 4:
 42; lane 5: 46

2.1. RAPD primer

Primer number	Nucleotide sequence (5' to 3')	GC content(%)
OPA-17	G A C C G C T T G T	60
OPA-19	C A A A C G T C G G	60
OPB-03	C A T C C C C C T G	70
OPB-09	T G G G G G A C T C	70
OPB-16	T T T G C C C G G A	60
OPF-16	G G A G T A C T G G	60

marker OPA-17 770 bp, OPB-03 1360 bp,
marker OPB-03 980 bp, OPA-19 1,300 bp,
6 marker OPA-17 1,340 bp, 41 marker
OPB-09 609 bp, OPF-16 1,115 bp, 51 marker OPA-17
580 bp (2.4, 2.5).
6 primer 87 가 band가 ,
primer 14.5 band가 . band 200-2,000 bp
가 ,
band pattern
가 .
6 OPA-17 primer 680 bp band
, 41 OPA-19 primer
2 band pattern (1,200 bp, 1,350 bp) . 51
6 , 41 , band
가 .
phylogenetic relation cluster 2.3
180 primer 174 primer monomorphic band
pattern 가 .
5. marker cloning
RAPD genetic marker 가 band GENECLEAN kit
pGEM-T Easy vector system cloning
marker OPA-17 789 bp (2.6), OPB-03 1,360
bp (2.7), marker OPB-03 749 bp (2.8),

OPA-19 1,300 bp (2.9), 6 marker OPA-17
 1,340 bp (2.10), 41 marker OPB-09 609bp (2.11),
 OPF-16 1,115 bp (2.12), 51 marker OPA-17 580 bp
 (2.13) cloning .

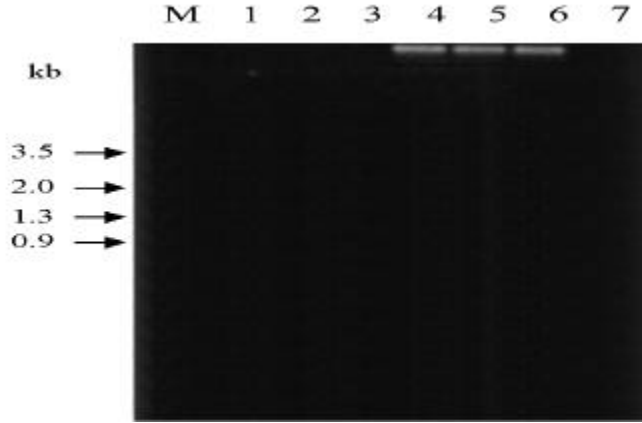


2.6. DNA OPA-17 PCR .

Lane M, DNA Hind -EcoRI DNA; lane 1-3:

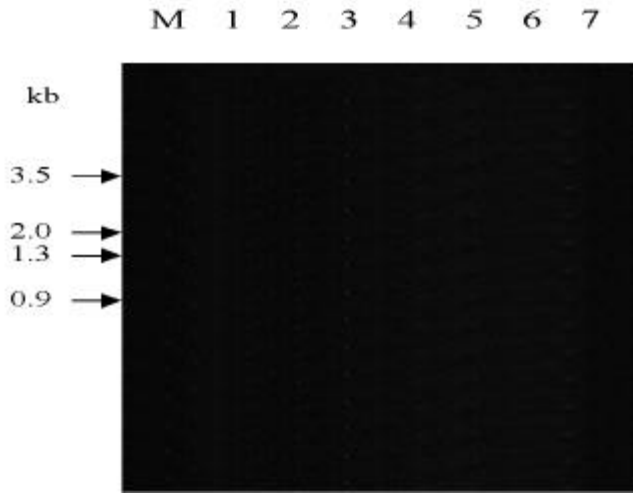
42, 52, 53; lane 4-6: 46, 54, 55. lane 7: EcoRI

plasmid DNA



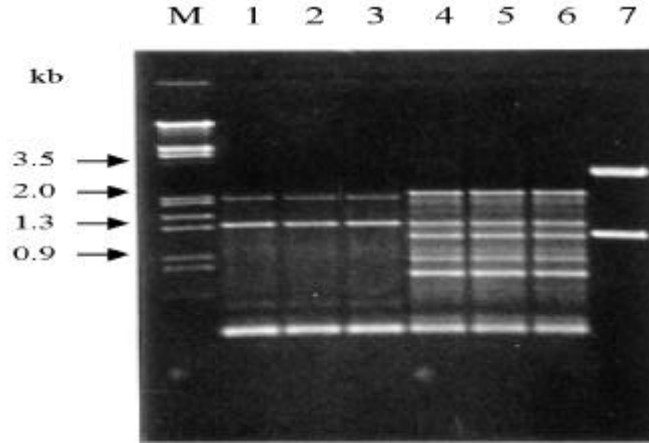
2.7. DNA OPB-03 PCR

Lane M: DNA *Hind* -*EcoR* DNA; lane 1-3,
 42, 52, 53; lane 4-6, 46, 54, 55; lane 7, *EcoR*
 plasmid DNA.



2.8. DNA OPB-03 PCR

Lane M: DNA *Hind* -*EcoR* DNA; lane 1-3,
 42, 52, 53; lane 4-6: 46, 54, 55; lane 7: *EcoR*
 plasmid DNA



2.9.

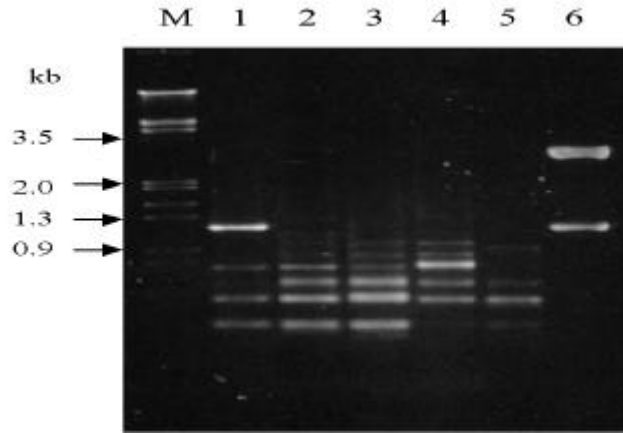
DNA OPA-19

PCR .

Lane M: DNA *Hind* -*EcoR* DNA; lane 1-3:

42, 52, 53; lane 4-6: 46, 54, 55; lane 7: *EcoR*

plasmid DNA



2.10.

6

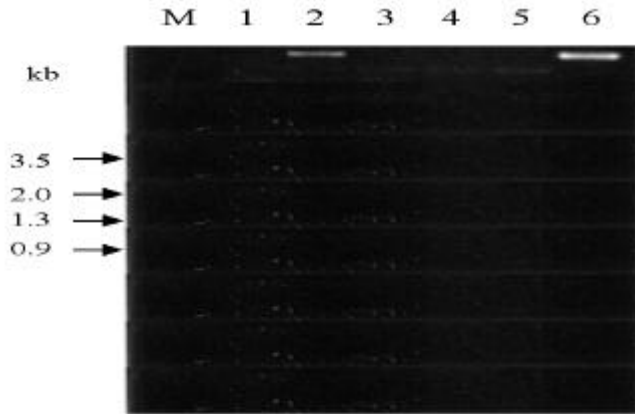
DNA OPA-17

PCR .

Lane M: DNA *Hind* -*EcoR* DNA; lane 1-3:

6, 41, 51; lane 4, 46; lane 5: 42; lane 6: *EcoR*

plasmid DNA

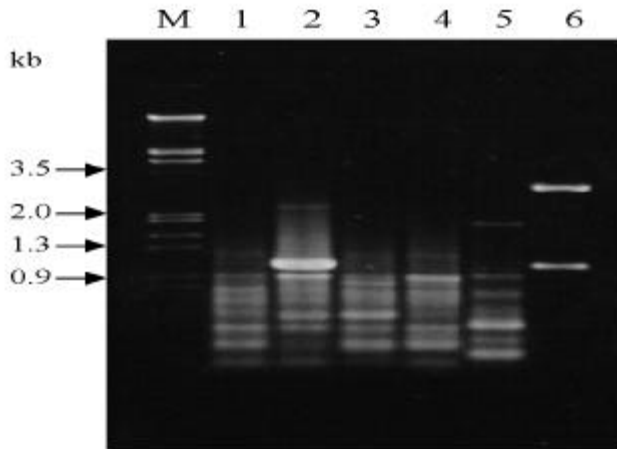


2.11. 41 DNA OPB-09 PCR .

Lane M: DNA *Hind* -*EcoR* DNA; lane 1-3:

6, 41, 51; lane 4: 46; lane 5: 42; lane 6: *EcoR*

plasmid DNA

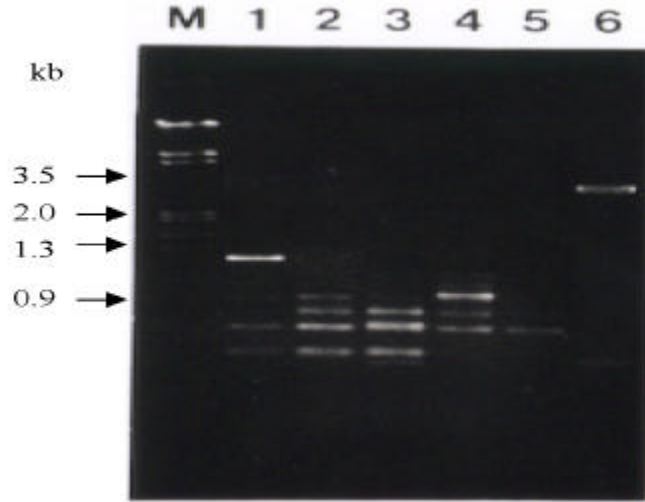


2.12. 41 DNA OPF-16 PCR .

Lane M: DNA *Hind* -*EcoR* DNA; lane 1-3:

6, 41, 51; lane 4: 46; lane 5: 42; lane 6: *EcoR*

plasmid DNA



2.13. 51 DNA OPA-17 PCR .

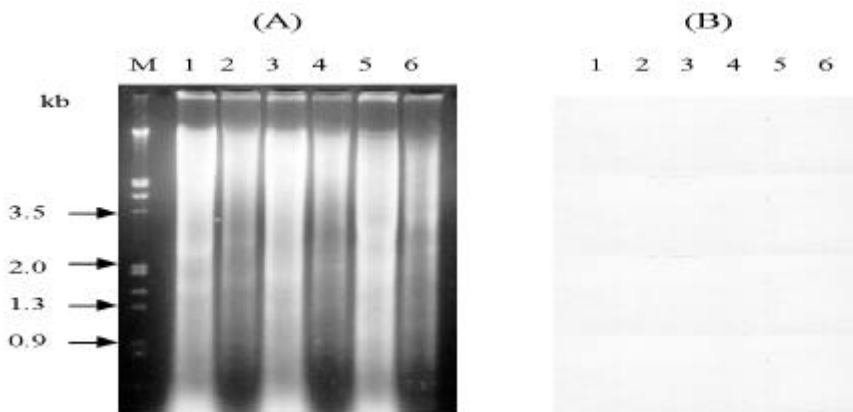
Lane M: DNA *Hind* -*EcoR* DNA; lane 1-3:
 6, 41, 51; lane 4: 46; lane 5: 42; lane 6: *EcoR*
 plasmid DNA

6. marker

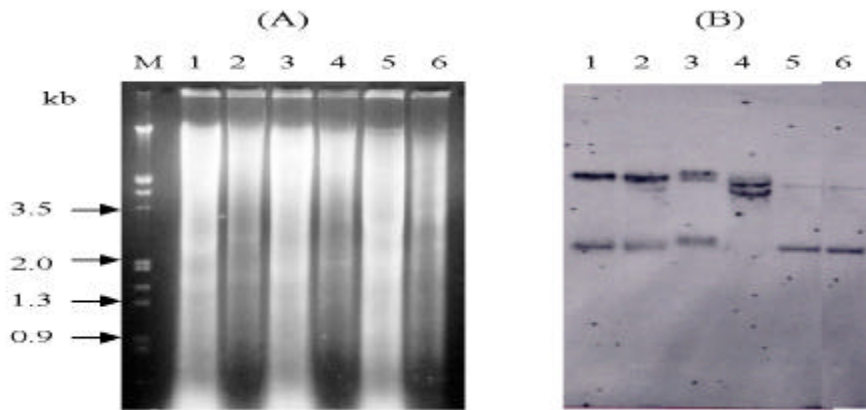
RAPD marker ,
 homology southern hybridization .
 marker가 cloning plasmid , *EcoR* probe

marker OPA-17 789 bp DNA
 hybridization , 2.14 band가
 genetic marker 가 , 가
 marker 가 . OPB-03 1,360 bp
 DNA probe 2.15
 marker 가 .

marker OPB-03 749 bp probe
 , genetic marker 가
 (2.16).
 6 marker OPA-17 1,340 bp
 hybridization , , marker
 가 (data not shown). 41 RAPD marker
 OPB-09 600 bp
 genetic marker 가 (2.17). OPF-16 1,100 bp DNA probe 41
 51 (2.18). 51 genetic marker
 OPA-17 580 bp probe hybridization
 , 2.19 51 .



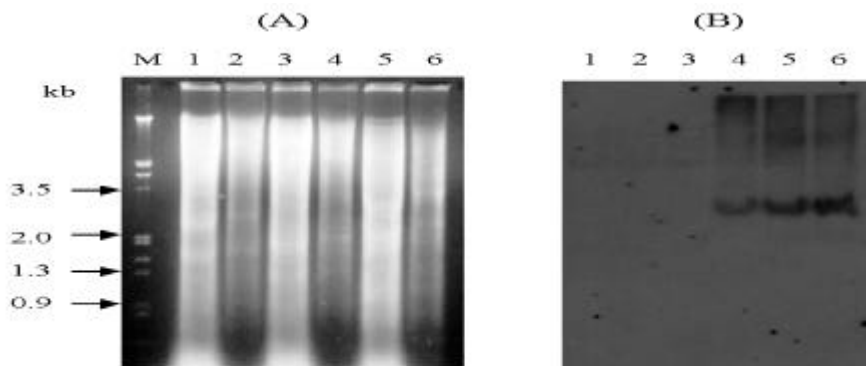
2.14. OPA-17 PCR
marker probe southern hybridization.
 Lane M: DNA *Hind* -*EcoR* DNA; lane 1-3:
 46, 52, 53; lane 4-6: 42, 54, 55; (A) DNA
Hind , 0.8% agarose ; (B) southern
 hybridization X-ray film



2.15. OPB-03 PCR

marker probe southern hybridization.

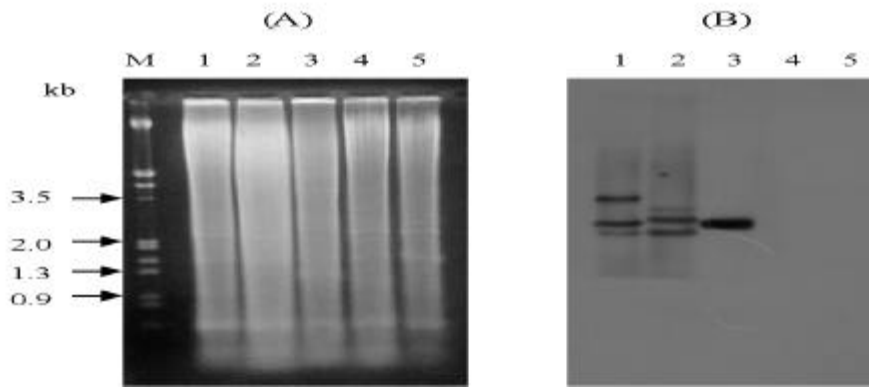
Lane M: DNA *Hind* -*EcoR* DNA; lane 1-3:
 46, 52, 53; lane 4-6: 42, 54, 55; (A) DNA
Hind, 0.8% agarose gel ; (B) southern
 hybridization X-ray film .



2.16. OPB-03 PCR

marker probe southern hybridization.

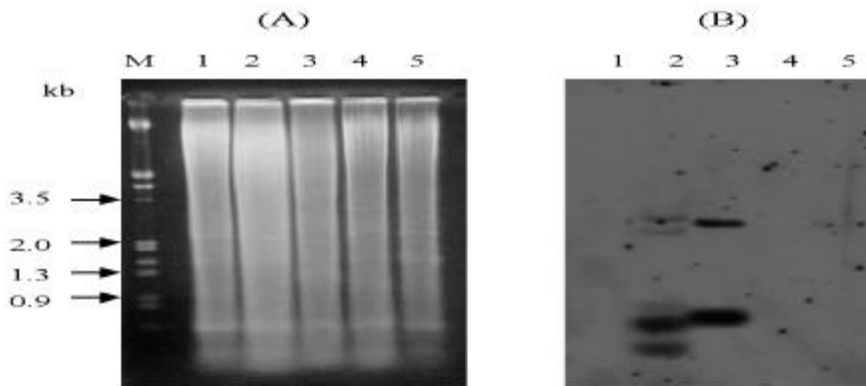
Lane M; DNA *Hind* -*EcoR* DNA; lane 1-3:
 46, 52, 53; lane 4-6: 42, 54, 55; (A) DNA
Hind, 0.8% agarose gel ; (B) southern
 hybridization X-ray film



2.17. OPB - 09 PCR 41

RAPD marker probe southern hybridization

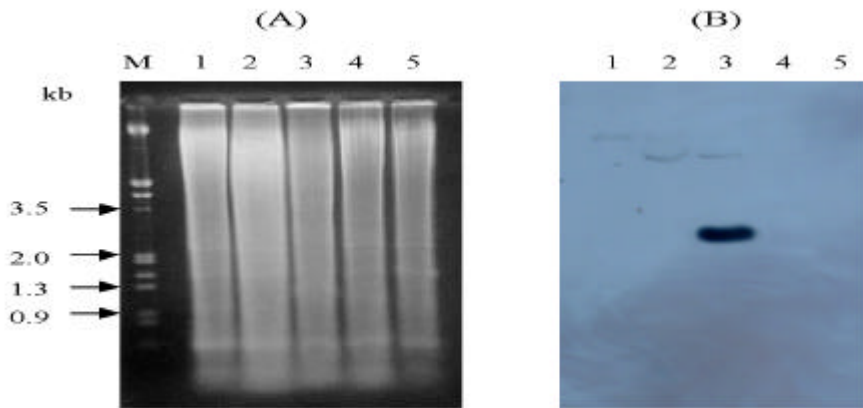
Lane M: DNA *Hind* -*EcoR* DNA; lane 1: 6;
 lane 2: 41; lane 3: 51; lane 4: 46; lane 5: 42;
 (A) DNA *EcoR*, 0.8% agarose gel
 ; (B) southern hybridization X-ray film



2.18 OPF - 16 PCR 41

RAPD marker probe southern hybridization

Lane M: DNA *Hind* -*EcoR* DNA; lane 1: 6;
 lane 2: 41; lane 3: 51; lane 4: 46; lane 5: 42;
 (A) DNA *EcoR*, 0.8% agarose gel
 ; (B) southern hybridization X-ray film



2.19. OPA - 17 PCR 41

RAPD marker probe southern hybridization.

Lane M: DNA *Hind* -*EcoR* DNA; lane 1: 6;
 lane 2: 41; lane 3: 51; lane 4: 46; lane 5: 42;
 (A) DNA *EcoR*, 0.8% agarose gel
 ; (B) southern hybridization X-ray film

7. kit 가

1) marker

Southern hybridization DNA

2.20 . 789 bp

open reading frame 가

homology .

DNA 749 bp

, DNA 가 ORF homology

(2.21).

	10	20	30	40	50	60	70	80
5'	GACCCTCT	TGTTGAACT	CTTTGGAAATA	ACCTAAGTCA	GTGAGGTTAG	ACTACTCTTG	GCATTTACAT	CAGCATGTGT
	90	100	110	120	130	140	150	160
	GTATAAGTTT	<u>CAGGAATTGA</u>	<u>GTTCACCTCA</u>	<u>TC</u> CATCAATA	TCACATGTAA	ATTCAATCAT	TATTTCTATT	TATGTTCTCT
	170	180	190	200	210	220	230	240
	TATAAATTATA	TAAAGTATCT	TACTGAAAAA	ATCTCTATAA	AATATTTGTA	AGTATCCAAT	ATTTGTTGAT	TGTATTGTTG
	250	260	270	280	290	300	310	320
	TGAAATCAAA	ATACTAAAAA	ATTGTGTTAT	CTAGTCAACG	TGAGTCAATC	TTCAGGTCCG	TGATCAAAAT	AGGCACGTAA
	330	340	350	360	370	380	390	400
	TGTCATCTAT	TTCTAGTTTT	GAGTCTTACA	TACTATATTA	GAGTATATTG	GATTCATTTG	GCATGTTGGT	TTTTGGAATT
	410	420	430	440	450	460	470	480
	TTATCTCATA	TTAAAAATTT	TGAAAAAATTG	GTGCAAGGCA	GTGCAACACT	CACITGCAAA	TGGFAGAACA	GTCATGGTGA
	490	500	510	520	530	540	550	560
	GCTTCGGTTT	CCTAAGCATA	AAAATAGGTT	GTTATGTTGG	TTTGGTCATT	<u>TGGAACCTCA</u>	<u>GAGACCAAGT</u>	<u>G</u> ACCCTTGCT
	570	580	590	600	610	620	630	640
	CCCCAACAT	GGTAGTTTAC	CTTCTTTGCT	TCTTGTGFTC	ACTTCCTTTC	GCTCTTTTCA	AGTTTTGTTG	CTAGGGTTTC
	650	660	670	680	690	700	710	720
	TATTGATTTT	CACTAATTTCT	ACCCTAGATT	GTTTTTCATA	GCTCTCAAG	CTTCTAAGAA	GTCAAAGTCC	TCITCGGGTA
	730	740	750	760	770	780		
	CCTATGATAC	ATGCAAGTTT	GTTGCCCTGC	GTGTCAAGCT	AGGCACAAAT	AATATGATCA	CAAGCCGTC	3'

2.20. marker .

	10	20	30	40	50	60	70	80
5'	GGACTGCAGA	CATCGATTAC	ATAACGAAAA	TCACCATAGA	AACCTCTCTT	TCTTATAGGC	GTACAAAGAG	GGTCCCATAG
	90	100	110	120	130	140	150	160
	ATCAGGATAT	AGGTTGAGAA	GCACTACGAA	GGACTACCGG	CAAAAGAGTA	AATCATCGAT	CGACTCAATT	CTTTCCTATC
	170	180	190	200	210	220	230	240
	TTGGTCTAGA	AAGAGAGCGG	CTGAAGTAAC	TGGCAATAGT	GCAGAAAAAA	GCTTTACCAT	CAAGCCGCTT	GCTTTTGTCT
	250	260	270	280	290	300	310	320
	CTGCCCTCTT	TTGTGAGAAT	TTATCGGTAC	CGTACACCAC	CTGAAGCGGA	GTCCGAGCTT	<u>GTGGCCCTAT</u>	<u>TCTTCACTGA</u>
	330	340	350	360	370	380	390	400
	GCACAGTGGG	CTGACTTTGC	TTTGTATGAT	TTTTCCTTGC	CCAAGACTGA	CATTTGTTTC	GTTTGAGTGC	ATGAAGTGT
	410	420	430	440	450	460	470	480
	GTTGTAAGTA	TGTGTAAGT	TAAAAATAAA	AGTAGATGGT	TGATGTTAGA	GAAGTCCCTT	CTATGATTCT	TTACCGAATC
	490	500	510	520	530	540	550	560
	GATGCTTTT	GAGTGTGTTT	GCTTTCACAA	GACAAGGGGA	TGTGCTCCCA	TCCCTCCGGA	GATCATAGGA	AAGATGGAGT
	570	580	590	600	610	620	630	640
	GATTCCTCAT	CTGGCTTCTA	CCTATTTAAG	AGAGTCGATA	TTTCACITTA	AAGACAGCTC	GTTGGCTAGC	TACCAGATAG
	650	660	670	680	690	700	710	720
	AATACAGTCT	TACCTGTCTT	AGCTTGATAG	AAACCTAGCT	ACAAAAGCGA	CTTCCCTCTG	TTGAAAATAC	CAGTGGAGAG
	730	740						
	<u>AGTAGCTGGT</u>	<u>GT</u> TGCTTACT	CTGCAGTCC	3'				

2.21. marker .

41 RAPD marker RAPD marker OPB-09 609
 bp 2.22, OPF-16 1,115 bp 2.23

. Southern hybridization , 가 609 bp

frame stop codon ORF가

. Homology

retroviral protease reverse transcriptase

retroviral molecular fossil .

Southern hybridization 3 51

580 bp 2.24 .

```

10      20      30      40      50      60      70      80
5' TCATCCCCCT GCCCTTTTCA AGCCTTCTTC TTCTCACATA AGATACACAT GCCTTAGACA ATTTTGGGAA GGCCAAAGCC
   90      100     110     120     130     140     150     160
TTAGGCAAGT TTGGGAGGTT TTCCTCAGTT CGCAGAAAA CGGACCCGAA CGGCAGGATT TTTGAAACAT GAGCTTTTCA
   170     180     190     200     210     220     230     240
TGAATTTTTT AGATTGCCCT TCGGCAATGG GTGGTTGCC ATTTTGATGC TCCATTATC GAACGGATCA TTTCAATTT
   250     260     270     280     290     300     310     320
TTTGGGTGGT CCCTTTAGGA TTCATCAAGA ATAGTCTCT TTTACGAAGA ACGAACCAGC CGACGGGTTT TTTCAAACG
   330     340     350     360     370     380     390     400
CATCTTTTTC TGAATTTTTT GAAGCATTGG ACTCCATGAG TGATTGGATC ATTTTGAAAT TTTCTACCCG TCGCCTGCCT
   410     420     430     440     450     460     470     480
TATGCTTAAA GTGCTTCTCA TGCCTTAGCC AATTTTAGAG GCTTTTGACC ATGGACATGG AGTGCCTCAT GACTAAGCAA
   490     500     510     520     530     540     550     560
ATTTTGCC   CCAACAATGG GATTCAACAA CTAAAAACA TTCTCAAAA TTCTTTTAAG ATGAAAGATT TAGGAGCATT
   570     580     590     600
AACTTATTTT TTGACTTAG AGCTATATAT TGATACACCA GGGGGATGA 3
  
```

2.22.

marker .

	10	20	30	40	50	60	70	80
5'	TGGACTGCAG	ACTGGCTTAA	TTCCGCATA	ATATAGTAGT	CAACATTGTA	TGATGCAGCA	ACAAGGCCA	CTTGAGAGCT
	90	100	110	120	130	140	150	160
	TGAATCGTAA	GCGCATGAA	GGTGTGAAG	GCGCTGTTCT	TGGTATAGCA	GTTTAGCTCG	AAGTTCATCA	AAGGTTAAGC
	170	180	190	200	210	220	230	240
	ACGAATTATT	GTTGGTGACC	GTCCTGATAA	AGGATTCATA	TTCTTCAGGG	AGTCTGCCA	GTGCATAGAT	CACCATGTCT
	250	260	270	280	290	300	310	320
	TTAGAAGAAA	GG AGAGTT	TATGGCTTCT	AGGGAATTCC	<u>CAATGGACCG</u>	<u>AAGATACGTT</u>	<u>CCCATGGGTT</u>	GGATCCCTTT
	330	340	350	360	370	380	390	400
	TTCAAAGATT	GAAATTTGAG	TTGAGATGTA	ACTCACGAGC	AGTAGCCTGG	TCTAAAAATC	TTTTTTACAA	GGCTAACCAA
	410	420	430	440	450	460	470	480
	AATATCACGA	CAGGTTGTCA	GTTTCATGAAC	CTCTTGAAGA	ATCTCTTTTG	TAAGAGTTGC	ATTGATCCAT	GATAGGAGCC
	490	500	510	520	530	540	550	560
	TTTGGTCTGT	CCGAACCCAA	GCCGAATATG	CAGGGTTTTG	TATGGCGTTG	CCATTGGCAT	AACAAATAAA	CTGAGGAGGA
	570	580	590	600	610	620	630	640
	CTGGGAAACG	TTCCGTCGAC	AAAGCCGAGA	AAAGAATTTC	AGATTATAAT	GTAGGAATTG	GGATTTCAAA	AGAAGATAAT
	650	660	670	680	690	700	710	720
	TGGTAGAGTC	AAGITTAAGG	GAAACAAGAT	GGGTGATGTT	TGGTGTAACT	ACCATAGGAT	TGGGGGTCTC	TTCTGTCTCT
	730	740	750	760	770	780	790	800
	GAAGAAGCAG	GCATGAGGGA	TTGATGAAGG	AGAAGCCATA	GTGTTGTCAA	AGCAGCTTGG	AATACTACAA	TCAGAGGCTC
	810	820	830	840	850	860	870	880
	CATAGCTTTC	CGTTTCTATC	TATCATAAGC	GGAAAGCGAC	CAAAAAATCC	CTCAAAGTGT	GGACAAGGC	TAACCTCGGT
	890	900	910	920	930	940	950	960
	AAGAAGATGT	TCGATGCAAG	TGCTGTGGAA	TGGAGAGAAA	AGATAGGACA	GAGAAGGAGA	AAGAGTGAAG	TTCTGGTCTT
	970	980	990	1000	1010	1020	1030	1040
	TCTACTAGGT	<u>CCTCCTTTCC</u>	<u>AGCCTTCAGA</u>	TCAACTCGTT	GGGGAAGGAA	TGTTCTGGTT	GTATTGAGC	CCTCTACCCG
1	1050	1060	1070	1080	1090	1100	1110	1115
	TGGCTTTGCC	TCGAAGCCCG	TGGAAGGTTT	AAAGCAATAA	ACTCAGGCCTT	CTTAACCTTC	TCCATCTGCA	GTCCA

2.23.

6 41

.

	10	20	30	40	50	60	70	80
5'	TGACCGCTTG	TGGTAAGAGA	GATCCTTCGC	AAGCCCTTTT	CGCTTCGCTC	AAGGGCCGAA	GCTCAGGAGG	ACATGCCCCG
	90	100	110	120	130	140	150	160
	TCCTTTTGAA	TGACTTCTGG	TTTTGCQCAA	GCCCTGTATT	TCCTTCGAAA	ACCATATCAT	TTTGTATTG	AGGGTGAAGC
	170	180	190	200	210	220	230	240
	CAACAGACTC	CGCAGTTTTT	TGCATTGCTA	TGTTCTTACC	<u>ACTCGTCCC</u>	<u>AGTTGAG</u> CGG	ACCCGATGCC	GGTTCCTTG
	250	260	270	280	290	300	310	320
	AGCAACTGCA	AACCTGTCAG	CAAAAGCCCG	CGGGCCAATC	CCAGACGCTG	ATATTGTGGA	TGTGTCCGGA	CGGGATCCGT
	330	340	350	360	370	380	390	400
	GTTTCCAAT	TTGCTTCTT	CATTGACCGA	GCAGATGCAA	TTTGGCGCAA	TGTTCCGTC	TGGTGC <u>CAACA</u>	<u>CCGACCAGAT</u>
	410	420	430	440	450	460	470	480
	<u>CAAGA</u> GACAG	CTCATATTCA	CTTGTGTCA	TGATGACGAG	ACGATTTTCG	GCCGTCATGT	ATTCTGTGCC	AAAGCCGGCG
	490	500	510	520	530			
	CGATGCATCT	TTGCAACTGC	GTCGCTTCT	TCGGTCCCTA	CAAGCCGTCA			

2.24.

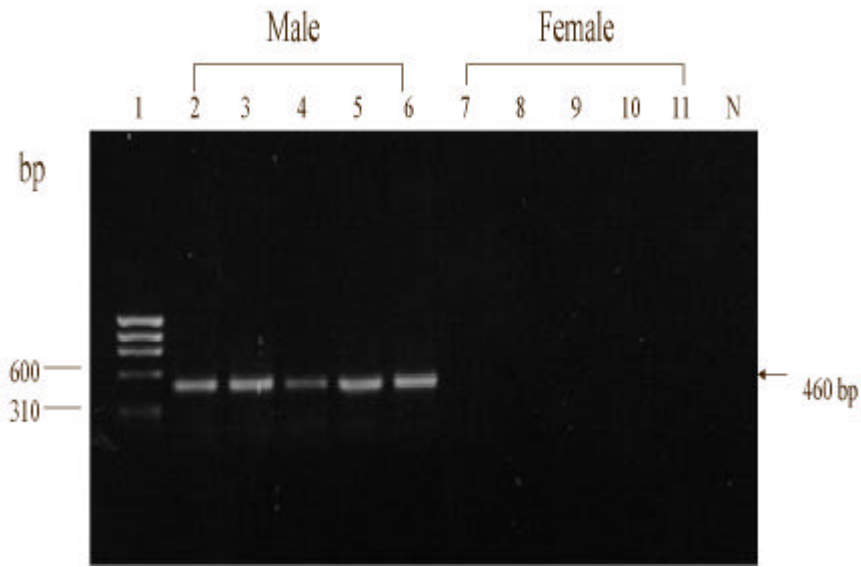
51

marker

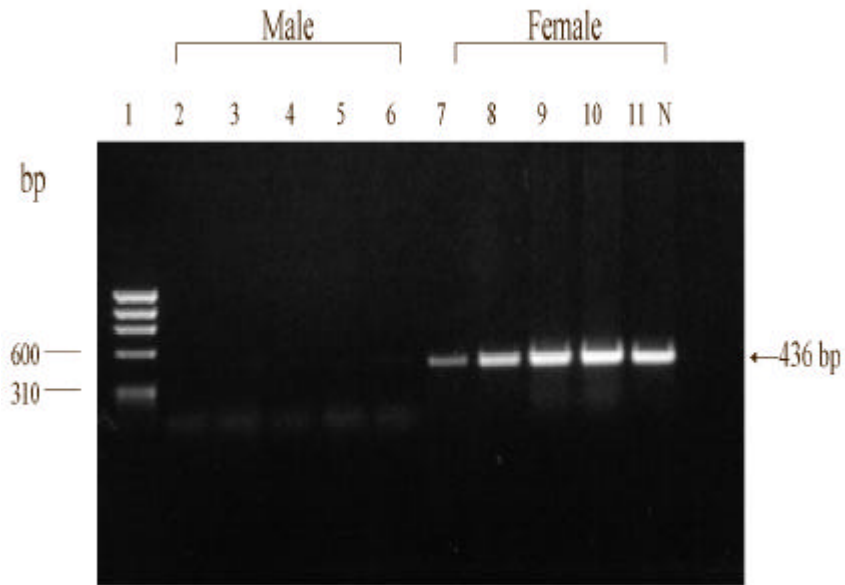
2) primer
 DNA
 PCR primer primer가 , ,
 (2.3).
 primer set PCR
 460 bp, 436 bp PCR .
 primer southern hybridization
 5 , 5 genomic DNA PCR
 primer 460 bp PCR
 (2.25). 가 primer
 436 bp DNA (2.26). primer
 PCR marker .

2.3. primer

	primer	primer	PCR product
male 1	F : 5'-GGAATTGAGTTCACCTCATC-3' R : 5'-CACTTGGTCTCTGAGTCC-3'		460 bp
female 1	F : 5'-CTTGTTGCCCTATTCTTCAC-3' R : 5'-AACACCAGCTACTCTCTCCA-3'		436 bp
41-1	mono 2-1 F : 5'-GCCATTTTGATGCTCCATTT -3 R : 5'-GTAGGGGGACCACATAGTT -3'		411 bp
41-2	mono 2-2 F : 5'-ATGGACCGAGATACGTT -3 R : 5'-TCCGAGCTTTCCTCCTGGAT -3'		684 bp
51	mono 3 F : 5'-ACCACTCGITCCCAGTTGAG -3 R : 5'-TCTTGATCTGGTCGCTGTTG -3'		207 bp



2.25. primer PCR .



2.26. Female primer PCR .

가

band .

genetic marker .

primer

DNA

PCR

(2.27).

3

(6, 41, 51). PCR

6

41

,

primer

PCR

(2.27, lane 1, 2), 51

primer

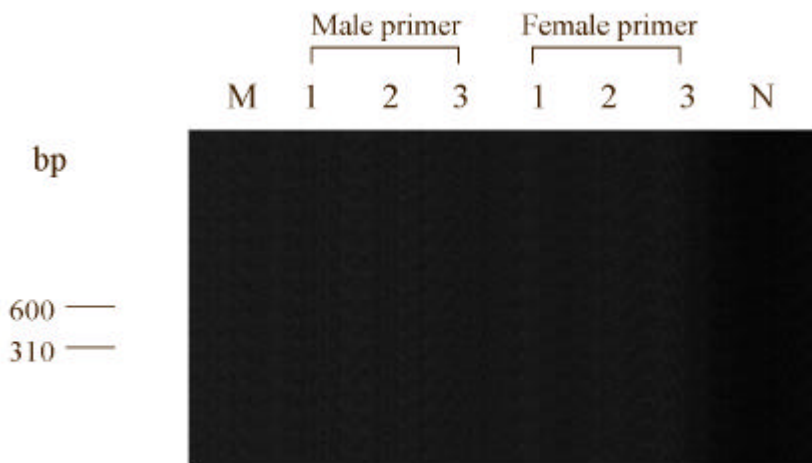
PCR

(2.27, lane 3).

51

가

가

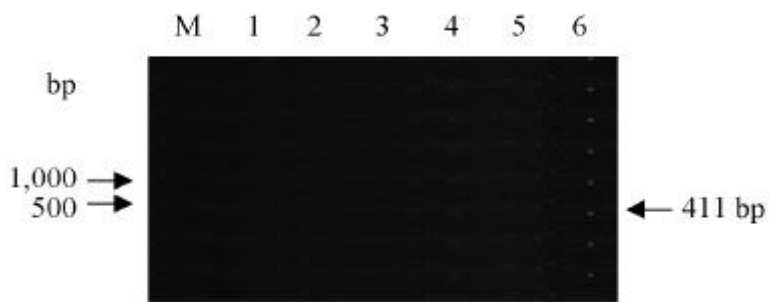


Monoecious plants

2.27. Male 1 primer Female 1 primer

PCR . Lane M: size marker, *Hae* X174 DNA; lane 1-3: 6, 41, 51 ; lane N,

41 RAPD marker 6, 41, 51
 609 bp primer (mono2-1)
 PCR 2.28 684 bp
 band가 . Southern hybridization ,
 41, 51 primer PCR
 , 884 bp band가 41, 51
 (2.29).



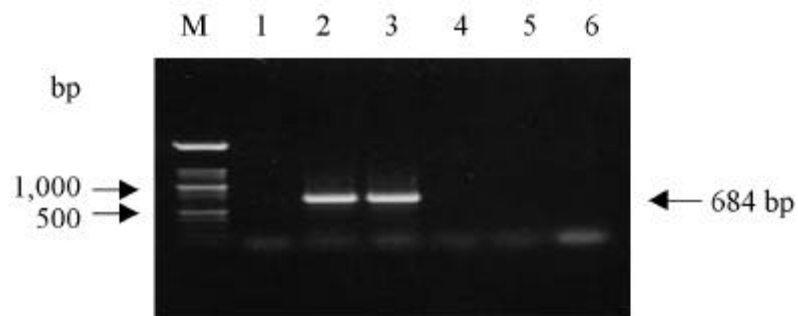
2.28. Mono 2-1 primer

PCR .

Lane M: size marker, 100 bp ladder; lane 1-3:

6, 41, 51 ;

lane 4-5: 46, 42; lane 6:



2.29. Mono2-2 primer

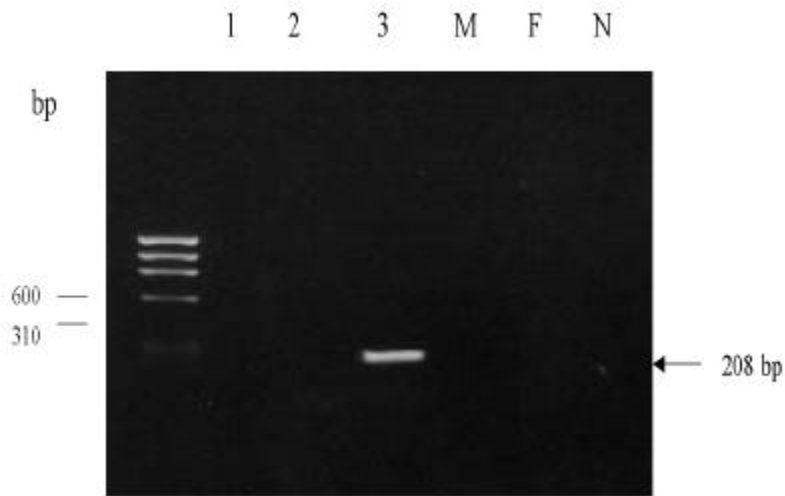
PCR .

Lane M: size marker, 100 bp ladder; lane 1-3:

6, 41, 51 ;

lane 4-5: 46, 42; lane 6:

3, 51 primer PCR, band가, 51, 207 bp band가 (2.30) 51 genetic marker 가 .



2.30. Mono3 primer PCR

Lane 1-3: 6, 41, 51 ; lane M: 46 ; F: 42 ; N:

RAPD southern hybridization

genetic marker OPA - 17 primer 770 bp, OPB - 03 primer 749 bp, OPB - 9 600 bp, OPF - 16 1,100 bp, 51 OPA - 17 580 bp . marker , primer . marker primer male 1 primer, marker female 1 primer

, primer mono2- 1, mono2- 2, mono3
 . primer PCR ,
 male 1 primer 460 bp band가 , female 1 primer
 436 bp band . marker
 mono 2- 1 primer 411
 bp band . mono2- 2 primer
 41 51 684 bp band가 .
 51 marker mono3 primer
 PCR , ,
 51 207 bp band가 .
 primer set
 , mono2- 1 primer PCR
 1 1.5

4

genomic DNA
Roggers Bendish (1988), Dellaporta (1983)
genomic DNA ,
DNA .
가 .
가 . RAPD southern hybridization
marker OPA-17 primer 770 bp, OPB-03 primer
749 bp, OPB-9 609 bp,
OPF-16 1,100 bp, 51 OPA-17 580 bp
marker ,
primer . primer , ,
genetic marker .
marker ,
,
,
.

4

1

anthocyanin (1982,) anthocyanin ,
 pH, , Ascorbic acid,
 가, “ (1986,)
 Ascorbic acid, , ,
 anthocynin, tannin 가, “ Alloxan 負荷家鬼
 (1987,) MeOH & ether
 mouse , 가, “
 (1989,), “ , , ,
 , , (1990,), “
 (1990,)
 rats alcohol , , microsomal protein , glycogen
 , glucose-6-phosphate dehydrogenase 가, “
 (1992,) 가 ,
 .
 ,
 , Schizandrin ,
 Kochetkov, N.K. (1961), Chen, Y.-Y. (1978) ,
 absolute configuration Chizhov, O.S. (1961), Ikeya, Y.
 (1978, 1979, 1982), Liu, C.-S. (1978) , Ben
 David Y. (1978), Ghera, E. (1978), Warshawsky, A.M. (1990) .
 Gomisin A , Taguchi, H. (1975),
 Chen, Y.-Y. (1976) , Taguchi, H. (1977),
 Ikeya, Y. (1979) , Nagai, H. (1989), Mizoguchi,

Y. (18991) . Gomisin D , Ikeya, Y.
(1979) , Lian-niang (1985) . Gomisin F
, Ikeya, Y. (1979) ,

Gomisin K1 , Ikeya, Y. (1978,
1979, 1980) , Suekawa, M. (1987) .
Gomisin N O , absolute configuration
Ikeya, Y. (1972, 1973, 1979, 1982) . Gomisin P ,
Taguchi, K. (1975), Liu, C.-S. (1978), Ikeya, Y. (1979)
, Ikeya, Y. (1980, 1981, 1982, 1983) ,
Hikino, H. (1984) . Gomisin Q ,
Ikeya, Y. (1979, 1990) ,

Liu, C.-S. (1982) .
Sesquicarene , Ohta, Y.
(1968), Bohlmann (1979) , Garbers, C.F.
(1975), Kitatani, K. (1976), Uyehara (1985), Johnson, C.R. (1987) .
Bisabolene , Bohlmann
(1974), Zdero, C. (1988) , Vig, O.P. (1979)

Chamigrenal Chamigrene , Ohta, Y.
(1968), Ito, S. (1967) , Tanaka, A. (1967),
Ireland, R.E. (1984), Martin, J.D. (1986) .
3-Copaene(=Ylangene) , Motl, O.
(1962), Irie, T. (1964), Ohta, Y. (1969) , Corey,
E.J. (1973), Wenkert, E. (1992) .
, Nigranoic acid Kikuchi, M
(1972), Schizandronic acid Takahashi, K. (1975, 1976), Schizandrol
Takahashi, K. (1976), Ayer, W.A. (1982)

, 2-Tridecanone Allen, R.R. (1965), Regnier, F.E. (1968)

, Majek, M. (1974), McDaniel, C.A. (1987)

, , 가 ,
1 2 ,

가, 가 , 日本, 中國

가 日本

가 anthocyan , 五味

, tannin, alcohol ,
alloxan 負荷家鬼 가

phenol ,
lignan gomisin, schizandrin,
sesquicarene, bisabolene, chamigrene, ylangene

, 가 .
 , , soft
 drink, , soft
 drink, extract 가 . ,
 가 가가 , 가
 가 가
 , 가
 , DATA ,
 , 가 route가
 가 가 .
 ,
 가 ,
 DATA , ,
 , , 呼吸
 困難, 急性炎症, 高血壓症, 動脈硬化症 ,
 가
 DATA , ,
 , ,
 , citral
 , total phenol phenolic acid
 , () ,
 lignan schizandrin,

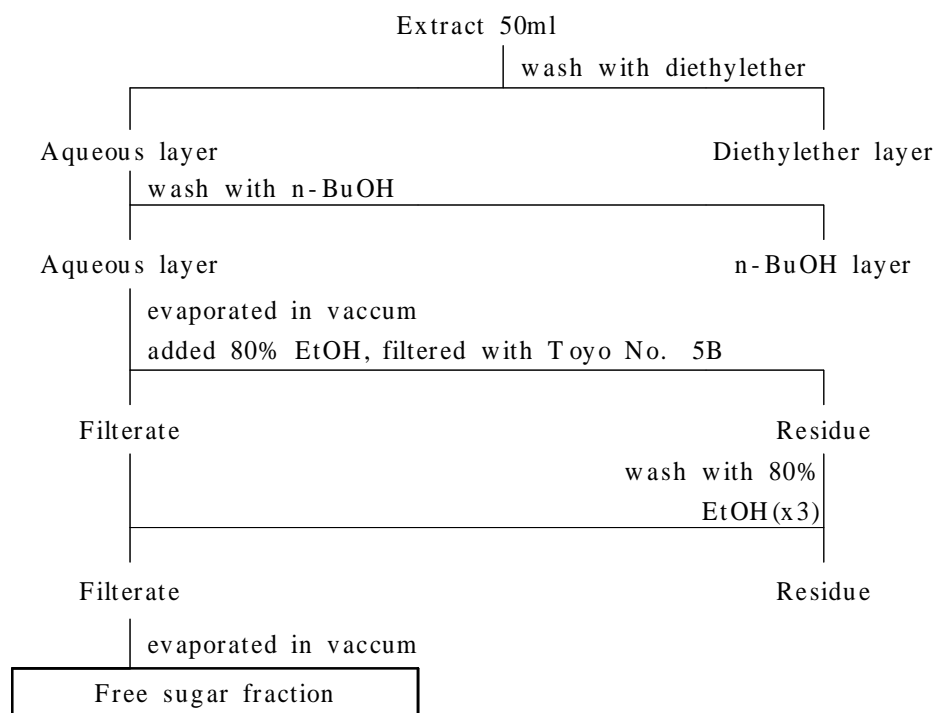
2

1.

2.

가.

- 1) , 10g .
- 2) 80% EtOH 50ml homogenizer(Nissei , Ace homogenizer AM-11, Japan) , 80 2 , Whatman No. 2 , 45 50ml 가 50ml가 EtOH . 가 . 4-1. .
- 3) C₁₈ cartridge . , C₁₈ cartridge MeOH , C₁₈ cartridge 가 4ml , 1ml membrane filter(Millipore, 0.45μm, Waters) 3.1. (HPLC) .



. 3.1. , .

3.1. HPLC

Instrument	Waters Associate
Column	Carbohydrate analysis (4mm x 30cm)
Solvent	Acetonitrile / Water (80:20, v/v)
Detector	Differential Refractometer (RI)
Flow rate	1.5ml/min
Injection Volume	20µl

.

1) HPLC

가) 10g 20ml homogenizer (Nissei , Ace homogenizer AM-11, Japan) , ,

3 . ehter 2
 , HPLC 40ml .
) C₁₈ cartridge , Membrane
 filter(0.45µm) 3.2. HPLC .

3.2. HPLC

Instrument	Waters Associate
Column	RSpak KC-811(4mm x 30cm)
Column Temp.	40
Solvent	0.1% H ₃ PO ₄ in water
Detector	Differential Refractometer(RI)
Flow rate	1.0 ml/min
Injection Volume	20µl

2) 가 (GC)

가) 1) HPLC 가) , 10ml

) ,
 BF₃/MeOH CHCl₃ 2ml 가 60 25
 ammonium sulfate 4ml 가 , CHCl₃ GC

) Hewlett packard 5890 series , Ultra 2 (Crosslinked MethylSiliconGum, 25m x 0.32mm x 0.52µm film thickness,

Hewlett Packard Co.), FID, 250 , 270 ,
 70 1 5 210

5 .

.

1)

가) 10g 100ml Homogenizer (Nissei, Ace
 homogenizer AM-11, Japan), 80 40 .
) 25% TCA (Trichloroacetic acid) 가 , 1
 , (5,000xg) .
) ethylether 가 , TCA, ,
 50ml가 0.2M Na-citrate
 (pH 2.2) .

2) : 가

가) 1g 6N HCl 10ml 110 24 가
 , .
) 0.2N sodium citrate buffer (pH 2.2) 10ml
 3.3. .

3.3.

Instrument	LKB 4150 ALPHA
Ion exchange resin	Ultrapac 11 cation exchange resin
Column Temp.	60
Buffer flow rate	45 ml/h
Ninhydrin flow rate	35 ml/h
Buffer pressure	22 bar
Ninhydrin pressure	16 bar
Reaction bath Temp.	130
pH range	3.2 10.0
Injection volume	40 μ l

. Phenol

1) Total phenol

가) Phenol phosphomolybdate
 Folin-Denis .

) Folin-Denis reagent : Sodium tungstate 25g, phosphomolybdate 5g,
 phosphoric acid 12.5ml 188ml 2
 , 가 250ml .

)

(1) 10g 95% ethanol 40ml ,
 homogenizer (Nissei , Ace homogenizer AM- 11, Japan) 3
 Whatman No. 3 (5) .

(2) 100ml mass flask 80ml, 1g ,
 Folin-Denis 2ml 5ml 가
 100ml .

(3) 1 , 660nm 3
 . blank test 0.1% 3.0% tannic
 acid spectrophotometer (Schimadzu, spectrophotometer UV - 120- 02,
 Japan) , standard curve total phenol
 tannic acid .

2) , ester

가)

(1) Krygier .

(2) , Soxhlet diethylether 12
 30g 250ml 70%

EtOH : 70% acetone = 1 : 1(v/v) 가 , sonicator 5

3,000 rpm 30 .

(3) 5 , 1,250ml 100ml ,
 100ml 가 50ml가 ,

(4) .

)

(1) 6N HCl pH 2 ,
4,000rpm 30 .

(2)

250ml hexane 5 .

(3) diethylether : ethylacetate = 1 : 1(v/v, DE/AE)
6 .

(4) DE/AE anhydrous sodium sulfate
30 , MeOH
10ml , 가

.

) Ester

(1) Sodium sulfate ,

(2) 가 4N NaOH 250ml 가 가 (가) 4 .

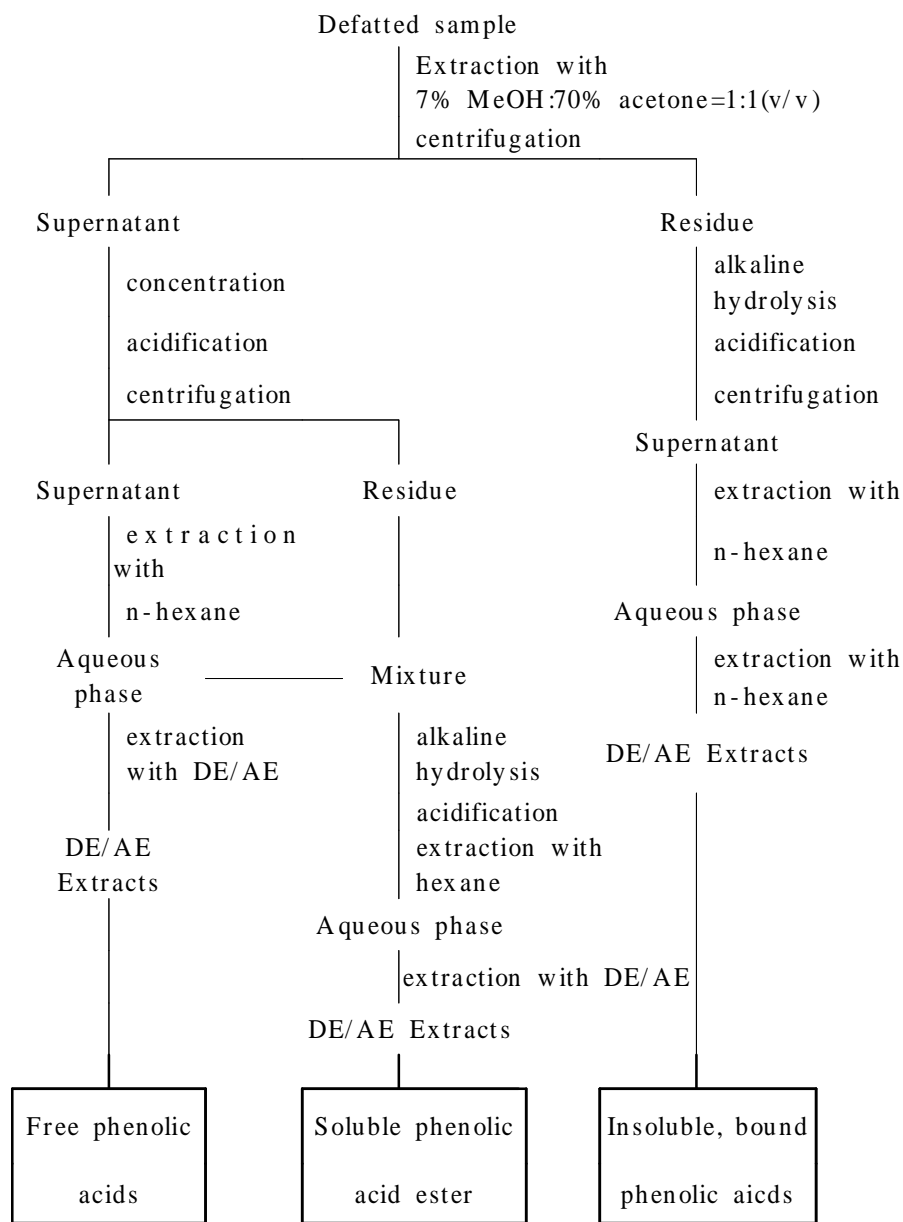
(3) 6N HCl 가 pH 2 ,
hexane) (3), (4) .

)

(1) {가} (4) 4N NaOH 250ml 가 , 가
4 가 .

(2) , 6N HCl 가 pH 2 ,
hexane DE/AE ,
MeOH , 10ml
가 .

. 4-2. .



. 3.2. ,

) (TLC)

(1) 10ml , Ester
TLC .

(2) Plate(silica gel F₂₅₄, Merck , 0.25mm) (1mg/ml)
, Ester
spotting toluene:ethylacetate:formic acid(5:4:1, v/v)
ferric chloride 5% potassium ferricyanide 0.5%
1:1 ,
HPLC GC .

) Phenol HPLC
10ml , Ester
1ml membrane filter(Millipore, 0.45µm, Waters) 3.4.
(1,000ppm) HPLC .

3.4. HPLC

Instrument	Waters Associate			
Column	µ Bondapak C ₁₈ (4mm x 30cm)			
Solvent system	Solvent A ; 100% CH ₃ CN Solvent B ; Water : CH ₃ CN : CH ₃ COOH = 86 : 8 : 4(v/v)			
Elution programming	0 - 10 min	solvent A	1 %	solvent B 99 %
	10 - 50 min	solvent A	60 %	solvent B 40 %
	50 - 55 min	solvent A	60 %	solvent B 40 %
	55 - 60 min	solvent A	1 %	solvent B 99 %
Detector	UV 280 nm			
Flow rate	1.5 ml/min			

) Phenol GC
(1)
(2g) 2ml Vial 가

- 2가 .
- (2) N,O-bis(trimethylsilyl)acetamide
- (가) N,O-bis(trimethylsilyl)acetamide 0.1ml 가 Vial
70 7 가 .
- () MeOH 가 2ml , GC .
- (3) 14% BF₃-MeOH
- (가) 14% BF₃-MeOH 1ml 가 Vial 2
100ml ,
- () NaCl 20ml ethylether 10ml
- () Ethylether 가 ethylether 2ml
GC .
- (4) GC 3.5 .

3.5. GC

Instrument	Hewlett packard 5890 series
Column	Ultra 1(Crosslinked MethylSiliconGum, 25m x 0.32mm x 0.52 μm film thickness, Hewlett Packard Co.)
Detector	FID
Carrier gas	N ₂
Detector & Injector	300
Temp.	
Oven Temp.	60 (1), 10 , 300 (15)

-
- 1)
- (가) 20g chloroform : MeOH : water = 2 : 1 : 1(v/v)
- Bligh Dyer .
- () , 20 (1,000ml) homogenizer(Nissei ,

Ace homogenizer AM-11, Japan) ,
 48 , chloroform
 24 , chloroform
 3 .
) Chloroform oven

24 , 가

2)

가) Rouser silicic acid (SACC) , .

) , silicic acid(100 200 mesh, Sigma , U.S.A)

MeOH 110 oven

24 .

) silicic acid 30g chloroform slurry 가

column chloroform ,

column 2 .(column size : 1.7 x 40 cm)

) chloroform column , chloroform

(), acetone() methanol() ²⁷⁾

)

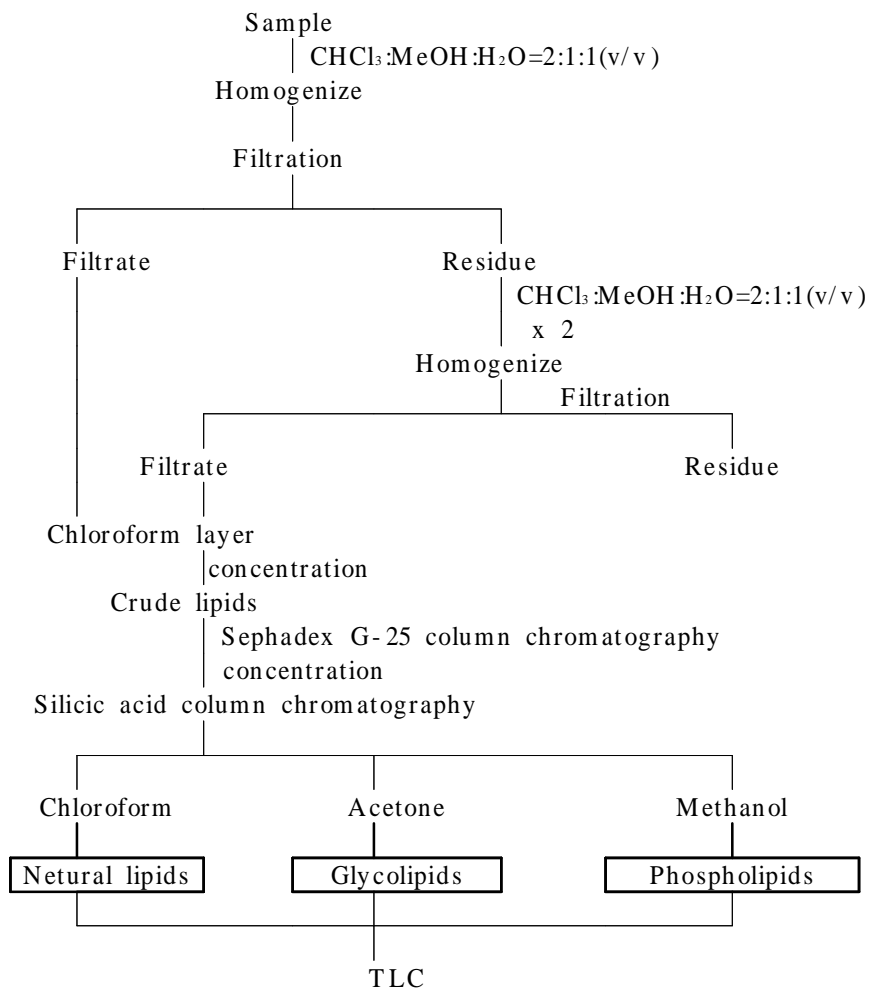
3.3. .

3) GC

가)

GC(Hewlett Packard 5890 series II)

GC 3.6. .



3.3.

3.6.

GC

Instrument	Hewlett packard 5890 series
Column	15% DEGS, glass, 3mm x 1m,
Detector	FID
Carrier gas	N ₂
Detector & Injector Temp.	250
Oven Temp.	80 (2), 5 , 180 (38)

- (1) ethylether 10ml 2ml
 가 0.5N NaOH in MeOH 8ml 가
 100 가 (5) , 5ml 14%
 BF₃-MeOH 가 2 .
- (2) NaCl 20ml flask 가 ,
 , 20ml petroleum ether (b.p 30 60) 가 1
 , ether 50ml
 (whatman No. 2) , 가 .
- (3) petroleum ether 1ml (2) GC
 . (500 ppm)

3.

가.

50g ethylether 50ml 3
 가 , ethylether 50ml

. GC/MS

1) GC/MS(Hewlett packard Co., 5970 series Mass Selective Detector)

2) : column Ultra 2(Cross-Linked 5% Phenyl Methyl Silicone, 25m x 0.32mm x 0.52 μm film), FID, 300 , 1 μl , Oven 60 1 , 7 100 100 1 3 160 , 10 10 280 10 .

4.

가.

1)

가) Hayashi , 100g 500ml 1% HCl in MeOH homogenizer , , Toyo No. 2 G₃ glass filter 3 .) 1% HCl in MeOH 100ml .

2) : 10g 0.01% HCl in MeOH 0.01% HCl in EtOH 20ml 가 Sonicator 1 3 , . , 440nm E₄₄₀/E_{MAX}

. Anthocyan

1) 0.1N NaOH 0.1N HCl 가 .

2) Anthocyanin

가) Aluminum chloride : AlCl₃ 5g ethanol 100ml .
) Ammonium molybdate : ammonium molybdate 2N

HCl pH 2 .

) Lead acetate : 1g lead acetate 75% ethanol 150ml

3)

Harborne Spectrophotometer
 , 5% AlCl₃ ethanol 3 가 ,

4) Anthocyanin

Fuleki Francis Philip .

TAN in mgs per 100g = OD X DV X 100/SV X TEV/SW X 10/E^{1cm}_{1%}

OD = , DV = , SV = , TEV = , SW =

$$E^{1cm}_{1\%} = \frac{\text{molar absorbance} \times 10}{\text{molecular weight}}$$

1) Amberite IRC-50

가)

(Amberite IRC-50)

5% HCl 500ml H⁺ , 500ml

0.1N NaOH 500ml 500ml .

5% HCl 500ml

H⁺ , 500ml 0.1N NaOH 500ml ,

500ml .

)
 (1) charge(, 100g
 MeOH 100ml 40ml charge)
 (800ml,) 0.3% HCl in EtOH
 (500ml,)
 (2) , (1)
 , 3
 (3) Whatman No. 1
 (PC) , 가

3.7.

3.7. PC

a	- -	15:82:3	
b	n - BuOH- -	4:1:5	
c	- -	5:2:3	
d	- -	30:10:3	
e	-	1:99	
f	EtOAc- -	80:15:15	
g	n - BuOH- pyridine-	6:3:1	
h	n - BuOH- pyridine-	6:3:3	
i	Benzene- -	2:2:1	
j	Benzene- MeOH-	90:16:8	

) PC

(1) Alkaline potassium permanganate : 2% Na₂CO₃ 1% KMnO₄
 100 ,

(2) AgNO₃ 0.1ml acetone 20ml 가 AgNO₃
 가 가 paper

0.5M-NaOH in EtOH .

(1), (5 10).

2) Aglycone

가) 가

(1) aglycone

4ml 50ml 1N HCl 20ml 가 30 가

(2) Aglycone

가 Amberlite IRC-50

300ml , aglycone 0.3% HCl in EtOH 200ml

, 4.7. PC (Whatman No. 1)

aglycone .

) Aglycone

(1) Whatman No. 3MM PC , aglycone

가 3.8.

(2) , aglycone Whatman No. 3MM

(20x20cm) banding / / (15:82:3) ,

aglycone 1% HCl in MeOH 가

, , .

3.8. Aglycone

PC

A	n-BuOH/ /	100:25:60	
B	n-BuOH/ /	4:1:5	
C	n-BuOH/2N HCl	1:1	
D	/	97:3	
E	/ /	5:2:3	
F	/ /	15:82:3	
G	/ /	30:10:3	
H	/	2:98	
I	/ /	5:1:5	
J	/	99:1	
K	EtOAc/ /	80:15:15	
L	n-BuOH/ /	7:2:5	
M	Acetone/ 10%	1:1	
N	Benzene/ /	2:2:1	
O	Benzene/MeOH/	90:16:8	
P	phenol/	4:1	
Q	isoBuOH/ /	8:2:3	
R	n-BuOH/Pyridine/	6:3:1	
S	Isoamylalcohol/ /	21:5:4	
T	Isopropylalcohol/ 10%	1:1	

.

1)

PC , aglycone spectrophotometer
(Hewlett packadr, HP 8453E UV-visible spectroscopy system)

.

2) HPLC

Whatman No. 3MM PC , aglycone

HPLC 3.9.

3.9. aglycone HPLC

Instrument	Waters Associate
Column	μ BondaPak C ₁₈ (4mm x 30cm)
Solvent	/ / MeOH(10:73:17)
Detector	546nm
Flow rate	1.0 ml/min
Injection Volume	10 μ l

5. : Schizandrin

가.

1) , 150g Soxhlet ether 8
MeOH 8

2) Ether ,
ether (Ether)

3) MeOH 200ml , EtOAc 200ml 3
MeOH 40ml , celite 16g
celite

4) Celite 30g column 3) Celite column
charge n-hexane 120ml, CHCl₃ 80ml, MeOH 120ml

5) n-hexane , ether

1) , n-hexane ether , Silica

gel(70 250 mesh, merck) 200g hexane slurry ,
column 3.10.

3.10.

No.			No.		
A	Hexane	500ml	G	Hexane-EtOAc(50:50)	500ml
B	Hexane-EtOAc(95:5)	"	H	100% EtOAc	"
C	Hexane-EtOAc(90:10)	"	I	EtOAc-MeOH(95:5)	"
D	Hexane-EtOAc(85:15)	"	J	EtOAc-MeOH(90:10)	"
E	Hexane-EtOAc(80:20)	"	K	EtOAc-MeOH(80:20)	"
F	Hexane-EtOAc(70:30)	"	L	100% MeOH	"

2)

가)

) Silica gel(70 250 mesh, merck) 30g n-hexane slurry
, column 4.11.

3.11.

No.			No.		
가	n-hexane	200ml		Benzene-Acetone (50:50)	200ml
	n-hexane-Benzene (1:1)	"		Benzene-Acetone (40:60)	"
	Benzene	"		Benzene-Acetone (30:70)	"
	Benzene-Acetone (90:10)	"		Benzene-Acetone (10:90)	"
	Benzene-Acetone (80:20)	"		Acetone	"
	Benzene-Acetone (70:30)	"		Acetone-MeOH (50:50)	"
	Benzene-Acetone (60:40)	"		MeOH	"

. Sephadex LH-20

- 1) Sephadex
LH-20(25 100 μ , Sigma) .
- 2) MeOH-CHCl₃(1:2, v/v) ,
(400ml) ,
charge , 80ml
, 10 .

• , Sephadex LH-20
schizandrin(Wako) TLC(silica gel
60F₂₅₄, merck , 10x10cm) spotting TLC
, benzene- acetone (9:1, v/v)
n-hexane- acetone(7:3, v/v) .

•
1) Sephadex LH-20 , TLC

2) TLC plate(20x10cm) banding n-hexane- acetone(7:3, v/v)
, *R_f*

MeOH .

3) 2) TLC 2) TLC ,
benzene- acetone(9:1, v/v) .

. HPLC

TLC membrane filter(Millipore, 0.45 μ m, Waters)

3.12. HPLC .

3.12.

HPLC

Instrument	Waters Associate
Column	μ Bondapak C ₁₈ (4mm x 30cm)
Solvent	acetonitrile/ anf (50:50)
Detector	UV 254nm
Flow rate	1.0 ml/min
Injection Volume	10 μ l

3

1.

가.

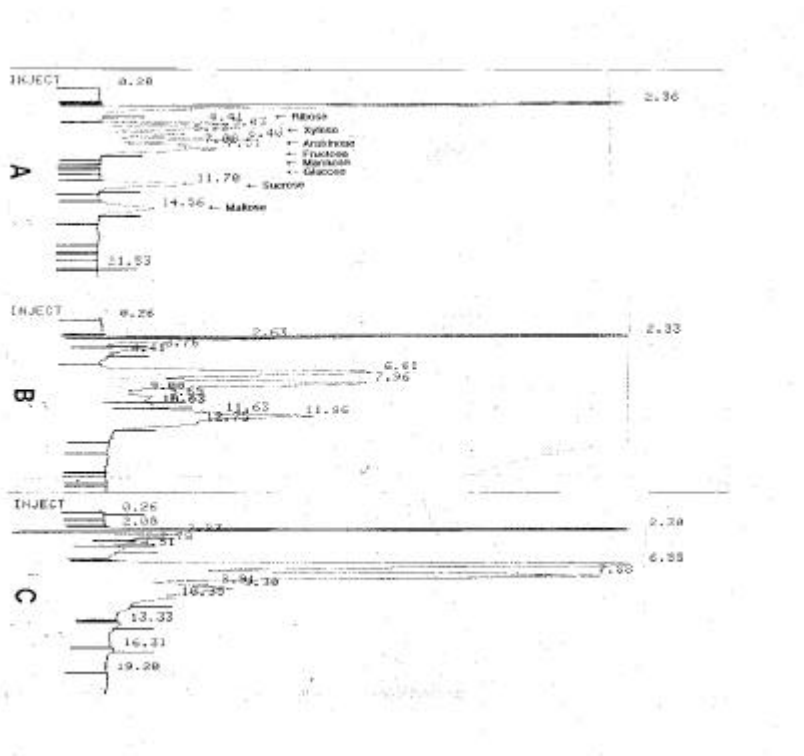
1) . 3.1.

HPLC 3.13. 3.4. .

3.13.

(: %)

	Ribose	Arabinose	Fructose	Glucose	Sucrose
	0.04	-	1.83	1.05	1.13
	0.08	0.01	3.71	2.51	0.02



. 3.4. , HPLC .

A : , B : , C : .

2) 3.13.

, sucrose
 , fructose glucose
 . sucrose
 , sucrose
 가 가 가

1) HPLC

가) 9 (2%) HPLC 3.14.

3.5.

3.14. HPLC .

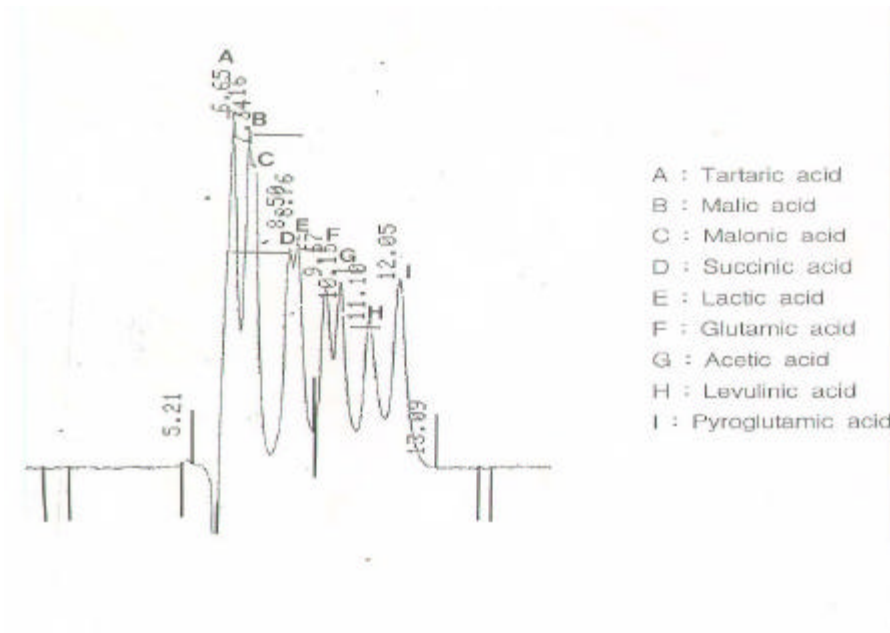
	()		()
Glutamic acid	9.67	Tartaric acid	6.65
Malonic acid	7.34	Succinic acid	8.50
Lactic acid	8.76	Acetic acid	10.15
Levulinic acid	11.10	Malic acid	7.16
Pyroglutamic acid	12.05	Maleic acid	6.70

) HPLC 3.15.

3.6.

3.15. HPLC ,

Citric acid	17.40 %	
Tartaric acid	4.68 %	
Maleic acid		4.94 %
Malic acid		14.20 %
Pyroglutamic acid	1.02 %	1.17 %



) citric acid가 가 , tartaric acid pyroglutamic acid가 . malic acid가 가 , maleic acid pyroglutamic acid가

3.15. 가

. , 3.6. retention time(Rt) 4.72, 6.05 8.32 peak가 Rt 8.32 succinic acid 3.41% . Rt 4.68, 6.11 8.06 peak가 , Rt 4.72 Rt 4.68 , Rt 6.05 Rt 6.11

6

) HPLC RI

가 가

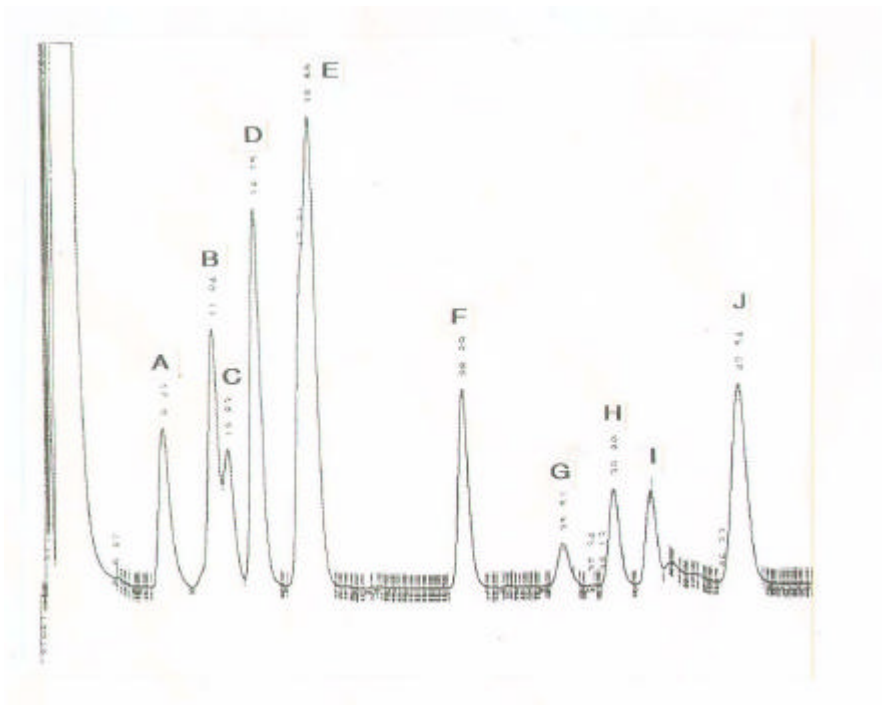
External
GC

2) GC

가) 10 (1,000ppm) GC

3.7. .

) Oxalic acid methylester Retention Time(Rt) 8.43, malonic acid methylester Rt 11.84, fumaric acid methylester Rt 12.83, succinic acid methylester Rt 14.75, maleic acid methylester Rt 18.60, glutaric acid methylester Rt 28.88, malic acid methylester Rt 35.51, tartaric acid methylester 39.00, citric acid methylester Rt 41.65, pyroglutamic acid methylester Rt 47.54 peak가 , malonic acid methylester fumaric acid methylester 가 .



3.7. GC

A : oxalic acid methylester, B : malonic acid methylester, C : fumaric acid methylester, D : succinic acid methylester, E : maleic acid methylester, F : glutaric acid methylester, G : malic acid methylester, H : tartaric acid methylester, I : citric acid methylester, J : pyroglutamic acid methylester.

) GC 3.16.
3.8.

3.16. GC

Malonic acid	0.3 ppm	2.8 ppm
Sunnic acid	247.2 ppm	21.6 ppm
Maleic acid	-	2.3 ppm
Malic acid	47,684.4 ppm	38,691.1 ppm
Pyroglutamic acid	1,241 ppm	101 ppm
Glutaric acid	74.3 ppm	-
Citric acid	11,939.1 ppm	3,330.5 ppm

) 3.16.

HPLC

, malonic acid maleic acid

가

가

1).

3.9.

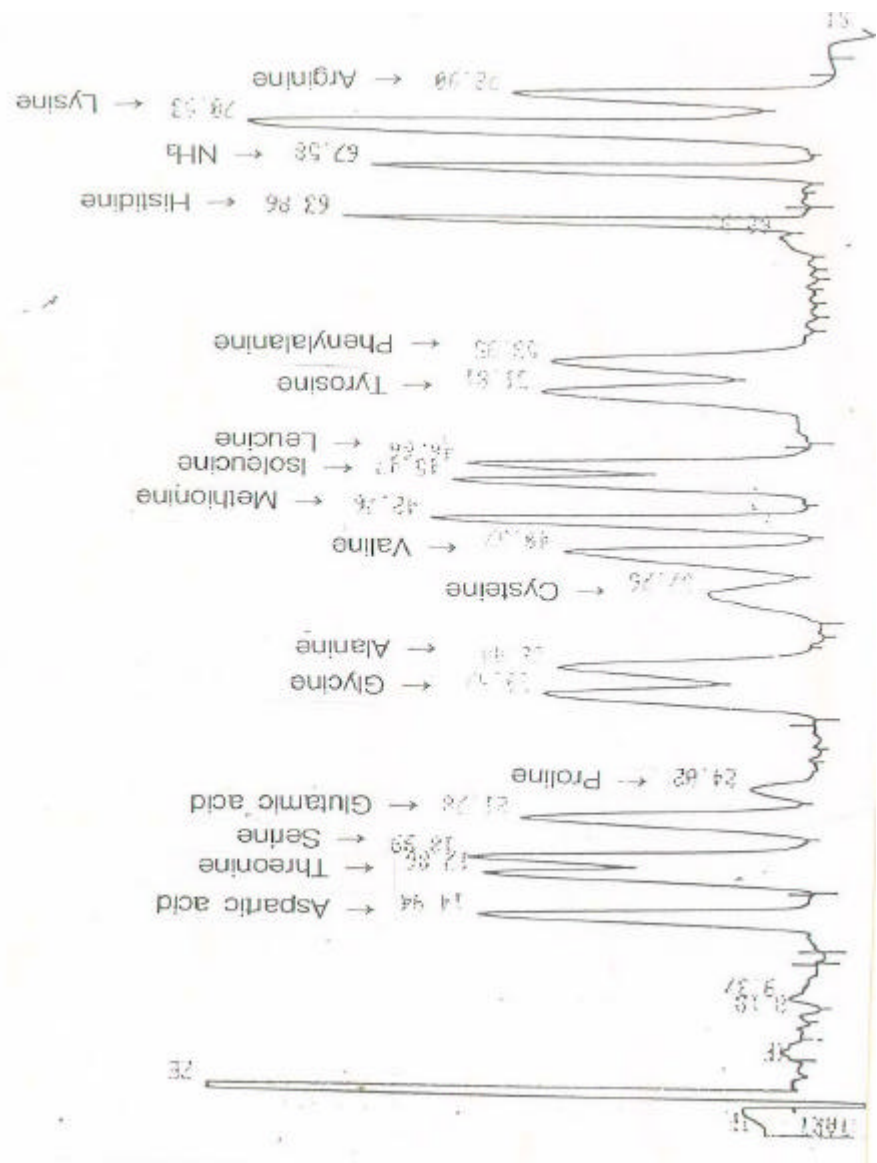
3.17.

2) lysine

. Lysine

300mg%

가



3.9.

3.17. ,

	(mg %)		(mg %)	
Aspartic acid	52.23	422.76	11.80	366.97
Threonine	154.73	511.36	31.41	427.55
Serine	0.00	600.01	0.00	491.94
Glutamic acid	97.37	721.95	11.40	677.03
Proline	23.42	427.13	5.42	239.04
Glycine	14.70	502.92	4.54	379.14
Alanine	50.19	348.16	7.14	273.84
Cystine	0.00	211.75	12.79	15.93
Valine	34.48	638.41	16.10	426.77
Methionine	21.58	148.35	0.00	35.93
Isoleucine	24.26	469.02	0.00	358.68
Leucine	31.10	653.56	0.00	447.41
Tyrosine	12.07	316.80	13.69	139.95
Phenylalanine	12.75	390.08	0.00	214.07
Histidine	120.30	518.55	40.40	371.96
Lysine	22.37	288.74	47.72	300.17
Arginine	50.37	408.26	0.00	430.43
Total	721.94	7,577.79	202.42	5,596.84

.

1)

Folin-Denis

tannic acid

, 1.275%,

1.560%

2)

TLC

가) 3.2.

Ester

TLC

3.18.

3.10.

3.18.

TLC

		CH	EP	CAT	TAN	PHC	CAF	GEFE	CI
		O	X	X	X	O	O	O	X
	Ester	O	X	X	X	X	X	X	X
		O	X	X	X	O	O	O	X
		O	X	X	X	O	X	O	X
	Ester	O	X	X	X	X	X	X	X
		O	X	X	X	O	O	O	X

* CH:chlorogenic acid, EP:epicatechin, CAT :catechin, TAN:tannic acid, PHC:phloroglucinol & coumalic acid, CAF:caffeic acid, GEFE:genticic acid & Ferulic acid, CI:cinnamic acid, O: 가 , X: 가 .

) 3.18.

3.10.

TLC

GC HPLC

3) GC HPLC

가)

HPLC

3.11.

3.19.

GC

3.12.

3.20.

, GC

가 HPLC

가

, HPLC

(gradient method)

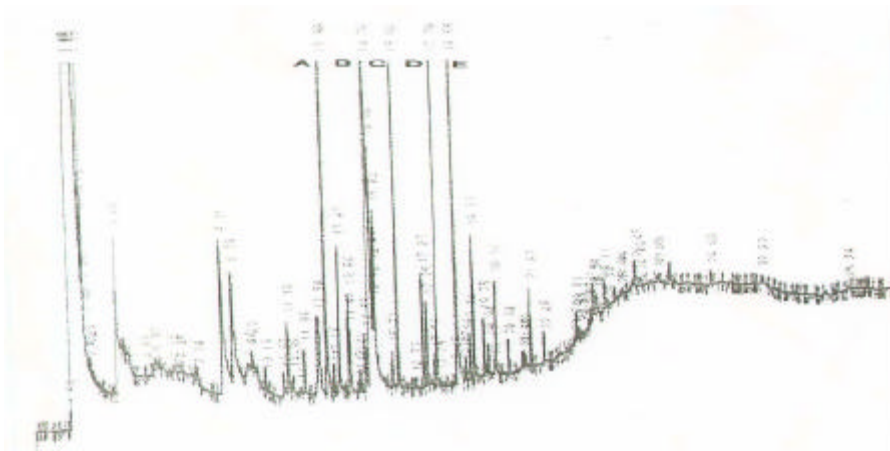
Rt 가

GC



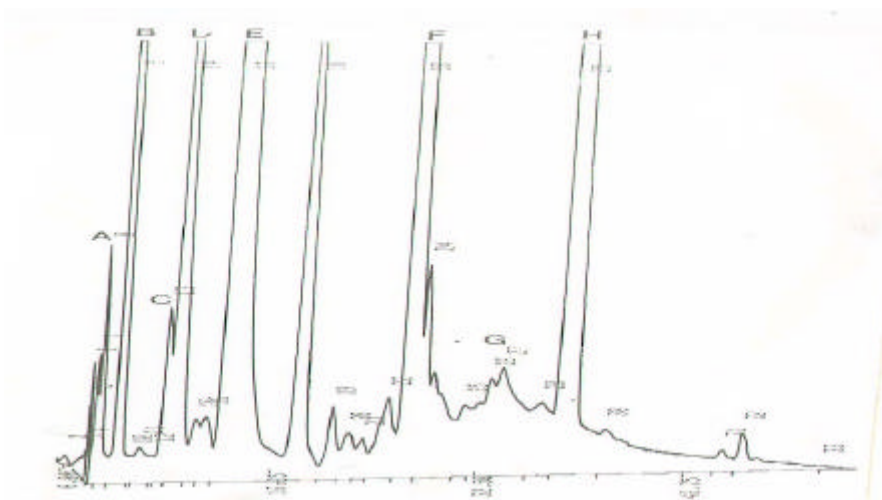
3.10. , TLC

A : , B : , C :
 , M : , D : ,
 E : , F : .



4.11. HPLC .

A : phloroglucinol, B : coumalic acid and gentisic acid, C : chlorogenic acid, D : catechin, E : epicatechin, F : ferulic acid, G : tannic acid, H : cinnamic acid.



3.12. GC (BF₃/MeOH)

A : cinnamic acid, B : gentisic acid, C : coumalic acid, D : chlorogenic acid,
E : ferulic acid.

3.19. HPLC .

	Rt		Rt
Phloroglucinol	3.215	Catechin	8.958
Coumalic acid	4.588	Epicatechin	13.175
Gentisic acid	4.588	Ferulic acid	27.001
Caffeic acid	6.408	Tannic acid	34.528
Chlorogenic acid	8.161	Cinnamic acid	39.555

3.20. GC .

	Cinnamic acid	Caffeic acid	Gentisic acid	Coumalic acid	Chlorogenic acid	Ferulic acid
Rt	12.90	14.42	14.76	15.98	17.70	18.55

* Caffeic acid N,O-bis(trimethyl silyl)acetamide

, 14% BF₃-MeOH .

) GC HPLC

3.21. .

) 3.21. cinnamic acid

, caffeic acid

가 . Gentisic acid

, coumalic acid

가

. chlorogenic acid ferulic acid

) 3.21. 3.18. , epicatechin, catechin,

phloroglucinol tannic acid 가 ,

chlorogenic acid, gentisic acid, coumalic acid, ferulic acid

. cinnamic acid TLC

3.21. .

(: ppm)

	CI	CAF	GE	CO	CH	FE
	22.71	46.73	28.14	trace	234.08	25.20
Ester	trace	-	17.20	trace	305.15	15.172
	33.00	trace	24.37	91.58	637.67	147.34
	1.84	-	96.76	58.44	167.71	9.36
Ester	trace	-	22.36	trace	357.97	4.06
	8.44	trace	18.53	46.70	302.04	10.77

* CI : cinnamic acid, CAF : caffeic acid, GE : gentisic acid, CO : coumalic acid, CH : chlorogenic acid, FE : ferulic acid

1)

가) Bligh Dyer silicic acid

3.22.

3.22.

(unit : mg/g. dry weight)

	110.5	32.0	3.0	145.5
	120.7	35.8	4.0	160.5

)

10%

가

GC

2) GC

가) 17 (500 ppm) GC

3.23.

3.13.

3.23. GC

	Rt (min)		Rt (min)
Caproic acid(C6:0)	2.08	Stearic acid(C18:0)	24.15
Caprylic acid(C8:0)	5.12	Linoleic acid(C18:2)	26.27
Capric acid(C10:0)	9.20	Linolenic acid(C18:3)	28.66
Lauric acid(C12:0)	13.31	Arachidic acid(C20:0)	29.33
Myristic acid(C14:0)	17.15	Eicosenoic acid(C20:1)	30.31
Myristoleic acid(C14:1)	18.08	Arachidonic acid(C20:4)	36.76
Palmitic acid(C16:0)	20.70	Behenic acid(C22:0)	38.80
Palmitoleic acid(C16:1)	21.36	Erucic acid(C22:1)	40.34
Oleic acid(C18:1)	24.83		

) , , ,

GC

3.24. 3.14. .

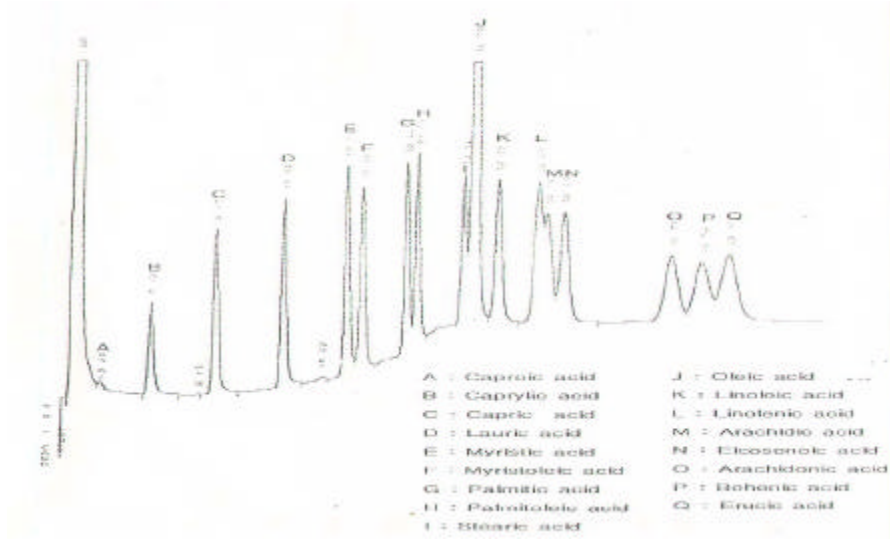
3.24. ,

(: ppm)

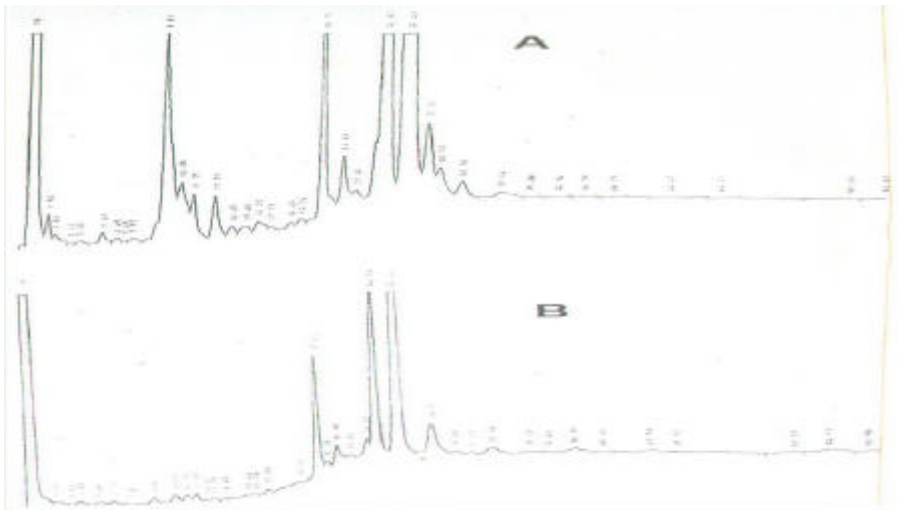
	A	B	C	D	E	F
Caprylic acid	50	14	3	169	25	2
Capric acid	1,795	14	9	2,178	29	5
Lauric acid	112	4	0.5	358	3	9
Myristic acid	1	0.5	0.5	8	10	16
Myristoleic acid	7	trace	14	3	6	trace
Palmitic acid	1,942	1,990	42	9,888	984	trace
Palmitoleic acid	trace	5	trace	trace	7	trace
Oleic acid	731	34	9	2,687	139	5
Linoleic acid	12,732	8,479	554	29,364	13,368	2,852
Linolenic acid	49	106	57	322	248	16
Eicosenoic acid	60	6	trace	131	3	2
Total	17,479	1,0652	689	45,108	14,822	2,907

A: , B: , C:

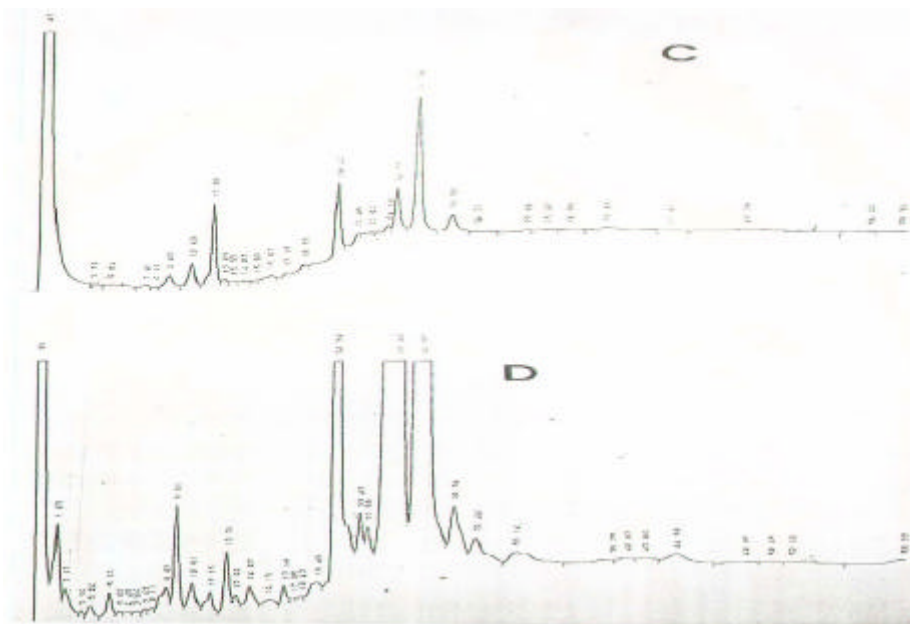
D: , E: , F:



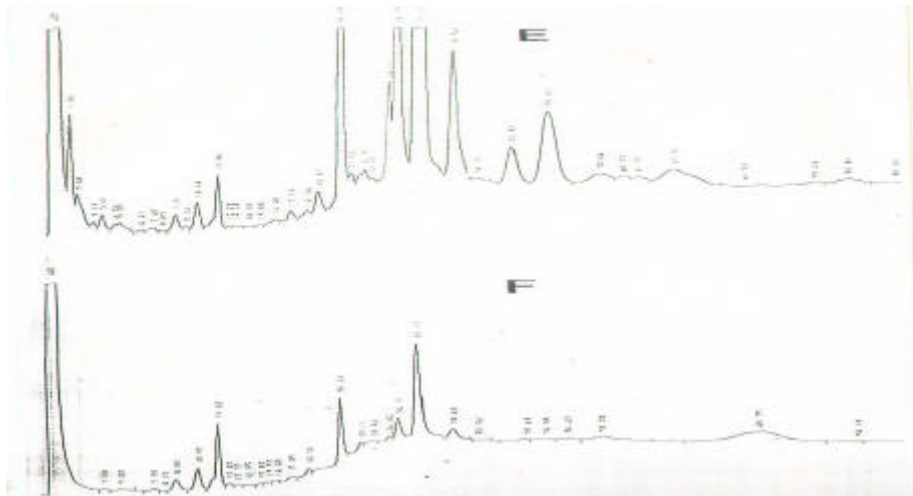
3.13. GC



3.14. , GC
A : , B :



3.14. , GC
 C : , D :



3.14. , GC
 E : , F :

) GC

oleic acid linoleic acid, linolenic acid

가 2

2.

가.

ethylether, GC/MS

3.15., GC peak MS

3.25. 3.16. 3.26. 3.17.

terpene monoterpene sesquiterpene

90%

가

3.15.

(R.T. 20)

3.25. 3.26. -ylangene,

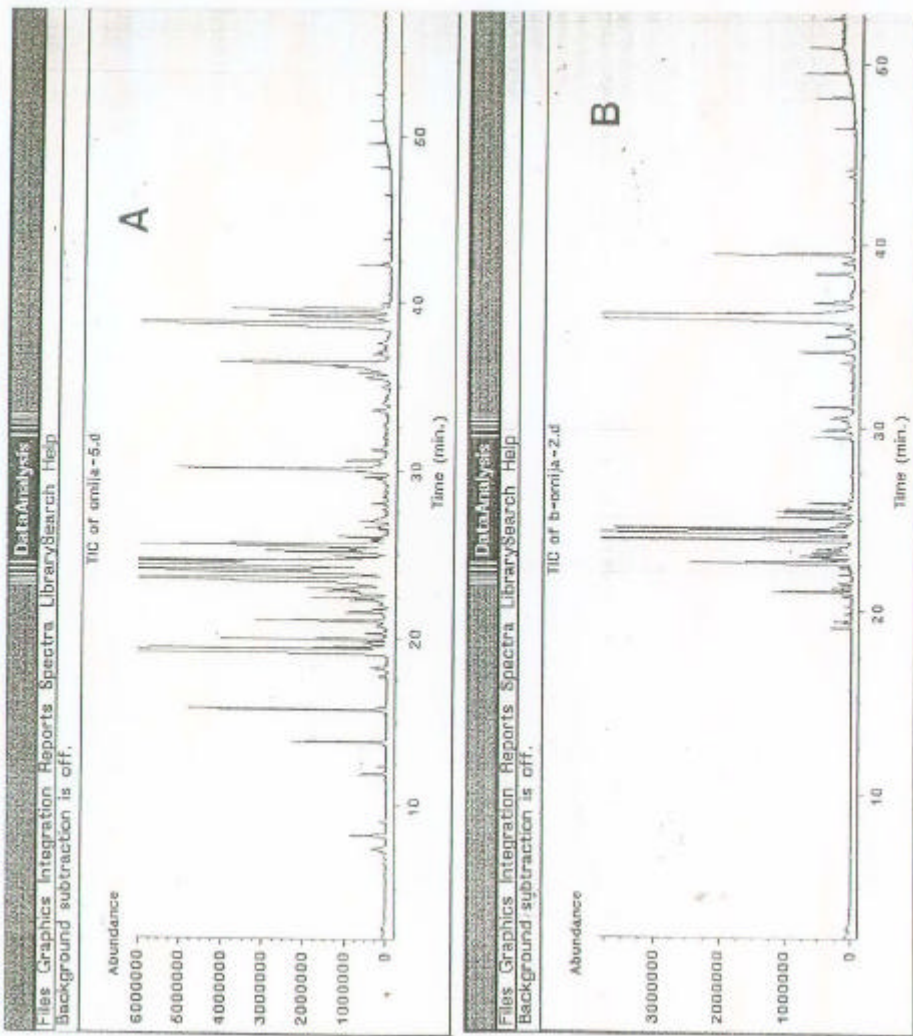
-elemene, -himachalene, -selinene widdrene

caryophyllene, calarene, cubebene, acoradiene

-himachalene

가

가



3.15. , GC

A : , B :

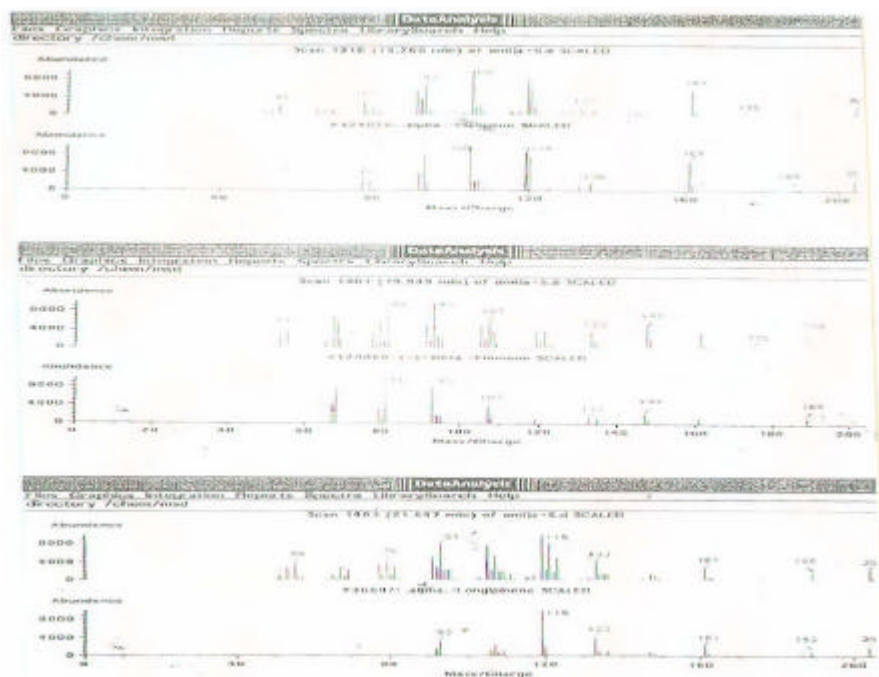
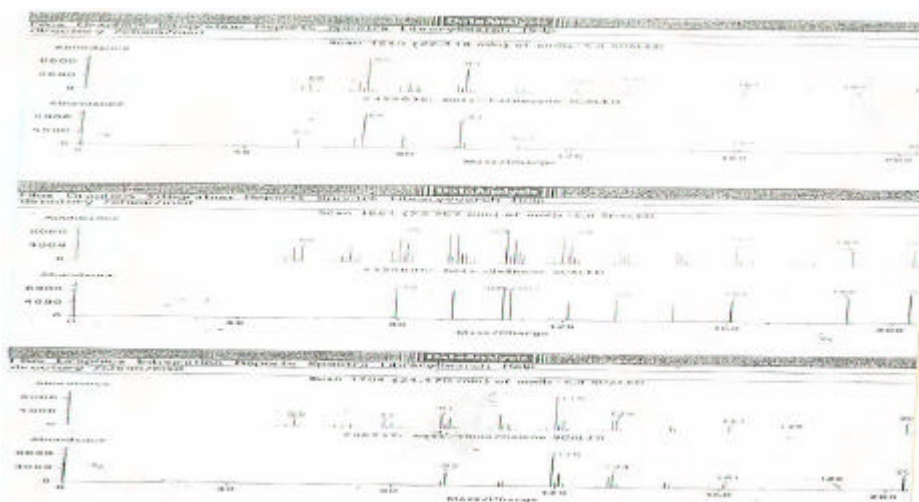
3.25.

GC/MS

Rt	Compounds	% Sg
7.437	Benzene, 1-methyl-4-(1-methylethyl)-	0.42
8.211	-Terpinene	0.69
11.857	3-cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-	0.34
12.315	1- -Terpineol	0.11
13.758	Methyl Thymylether	1.07
17.749	-Terpinolene	0.12
18.263	5H-Inden-5-one, octahydro-, trans-	0.30
19.296	-Ylangene	13.19
19.473	-Guaiene	0.78
19.707	1H-Pyrrole, 1-butyl-	0.40
19.944	(-)- -Elemene	1.81
20.305	1,2-Naphthalenediol, 2-ethyl-1,2,3,4-tetrahydro-1-methyl-, ci	0.26
20.738	Tricyclo[2,2,1,0 ^{2,6}]heptane, 1,7-dimethyl-7-(4-methyl-3-pentyl)-	0.11
21.052	-Elemene	1.88
21.554	-Longipinene	0.58
21.740	-Terpinene	0.13
22.045	Longipinene	0.14
22.274	(+)-Aromadendrene	0.55
22.415	-Farnesene	0.93
22.713	-Cadinene	0.54
22.828	Acoradiene	0.86
22.959	(-)- -Acoradiene	0.19
23.478	-Selinene	7.23
23.768	-Selinene	0.34
24.176	Naphthalene, 1,2,3,4,4a,5,5,8a-octahydro-7-methyl-4-methylene	0.82

3.25.

Rt	Compounds	% Sg
24.469	- Himachalene	10.05
24.590	Widdrene	5.53
25.173	- Cadinene	1.52
25.286	6,10,11,11- Tetramethyl- Tricyclo[6,3,0,1E2,3] Undec- 1(7)ene	0.57
25.761	- Muurolene	0.16
25.835	- Humulene	0.19
25.978	Quinoline, 2,6- dimethyl-	0.52
26.444	2- (5- Methyl- 3- Isoxazolyl)- 4- Acetyl- 5 - Methylpyrrole	0.12
26.638	Nerolidol	0.28
27.943	2- (1',1'- Dideutero- Allyl)Aniline	0.23
29.910	Bicyclo[4,4,0]Dec- 1- En,2- Isopropyl- 5- Methyl- 9- Methylene-	0.41
30.110	Cycloheptene,5- ethylidene- 1- methyl-	4.22
30.587	T - Muurolol	0.75
33.591	(+)- Oxo- - Ylangene	0.40
35.010	Azocine, 2- methoxy - 8- methyl-	0.15
35.552	2,3,4- T rimethylbenzaldehyde	0.58
35.844	2- Vinyladamantane	0.63
36.112	(E)- And(z)- 7- Methyl- 5,7- Octadien - 1- Ol	1.27
36.382	Cycloisolongifolene	4.54
36.877	2,2- Diisobutenyl- 3- cyclohepten - 1- one	0.32
37.158	2- Naphthalenecarboxylic acid,8- ethenyl- 3,4,4a,5,6,7,8,8a - oct	0.22
37.584	4(1H)- Quinolinone, 3- methoxy - 1- methyl-	0.29
38.709	Benzenemethanol,ar,ar, - trimethyl-	8.15
39.149	- 4- Carene	2.05
39.562	Phenol, - (2- methylallyl)-	2.67
42.262	4- (2'- ethyl- 5'- phenyl- pyrro- 3'- yl)pyridine	0.25
46.429	Tetracosamethylcyclododecasiloxane	0.05
50.854	1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15- Hexadecamethyl- octasiloxane	0.10



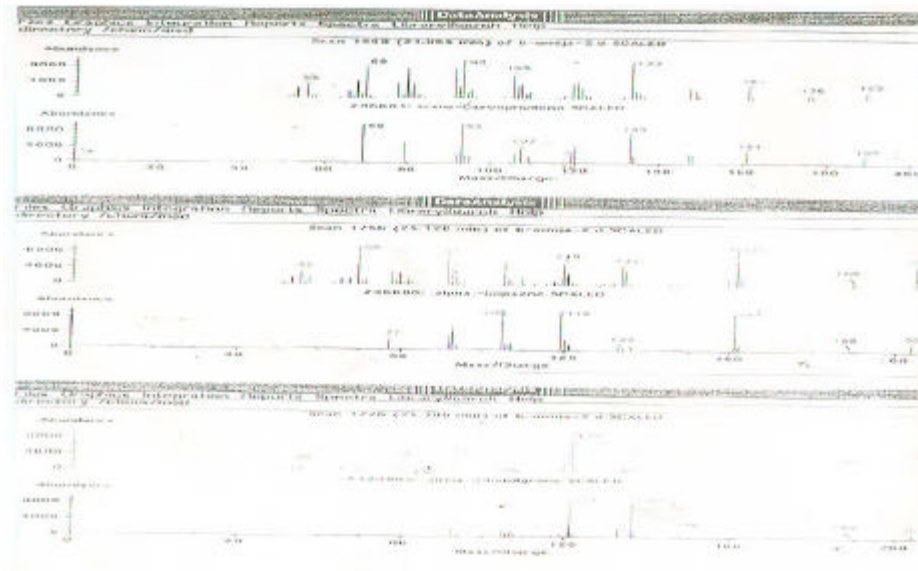
316.

MS

3.26.

G/MS

Rt	Compounds	% Sg
19.442	Selina-3,7(11)-diene	0.36
21.073	trans-Caryophyllene	1.65
21.255	1H-3a,7-Mrthanoazulene, 2,3,4,7,8,8a-hexahydro-3,6,8,8-tetram	0.51
21.542	-Longipinene	0.31
22.028	Tricyclo[3,3,0,0E4,6]octan-3-one,6-methyl-	0.30
22.661	Calarene	3.58
22.767	-Bisabolene	0.49
23.031	-Cubebene	1.49
23.247	-Chamigrene	0.96
23.385	Longipinene	1.56
23.955	(-)-Acoradiene	11.52
24.337	-Himachalene	10.19
24.519	1,2,2-trimethyl-1-(p-tolyl)-cyclopentane	5.74
25.129	-Copaene	1.74
25.390	-Chamigrene	1.67
25.911	N-(p-tolyl)-acrylic acid amide	1.14
29.516	-Selinene	0.96
29.893	(+)-Isobicyclogermacrene	1.05
31.143	Caryophylla-2(12),6-dien-5-one	1.09
33.588	Cyclopentene,1,3-dimethyl-2-(1-methylethyl)-	0.52
35.024	Aristolone	1.08
36.152	2-Naphthalenecarboxylic acid, 8-ethenyl- 3,4,4a,5,6,7,8,8a-oct	32.88
36.267	Benzene,1,4-dimethyl-2,5-bis(1-methylethyl)-	3.68
36.854	Tricyclo[3,2,1,0E2,8]Octan-7-one, 6-methyl-6- (2-methyl-2-prop	1.46
38.457	1,2,3,3a,5,6,6a,7-Octahydro-1,3a,6-trimethyl- 4H-cyclopent[d]i	1.40
39.496	2H-1-Benzopyran, 3,4-dihydro-2-methyl-	4.35
46.430	Benzeneacetic acid, 3,4-tris[(trimethylsilyloxy)-t	0.20
50.857	Tetracosamethylcyclododecasiloxane	0.40
52.420	1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-octasiloxane	0.28



3.17. MS .

3.

가. Anthocyan

1)

가) , 가 anthocyan

3.27. .

3.27. , 가 .

		0.1N NaOH	2N HCl	AlCl ₃
	Ammonium Molybdate	Vanillin	Lead acetate	FeCl ₃ -K ₃ Fe(CN) ₆

) , 0.1N NaOH 가 ,
 , 2N 가 .

) Vanillin ,
 . 4-27. ,

) anthocyanin 3.18.

B OH 가 cyanidin, delphinidin, petunidin
 aluminium chloride , ammonium molybdate , lead acetate 가
 , , OH 가 pelagonidin,
 peonidin, malvidin 가
 anthocyanin . 3.27.

pelagonidin, peonidin, malvidin ,
 cyanidin, delphinidin, petunidin .

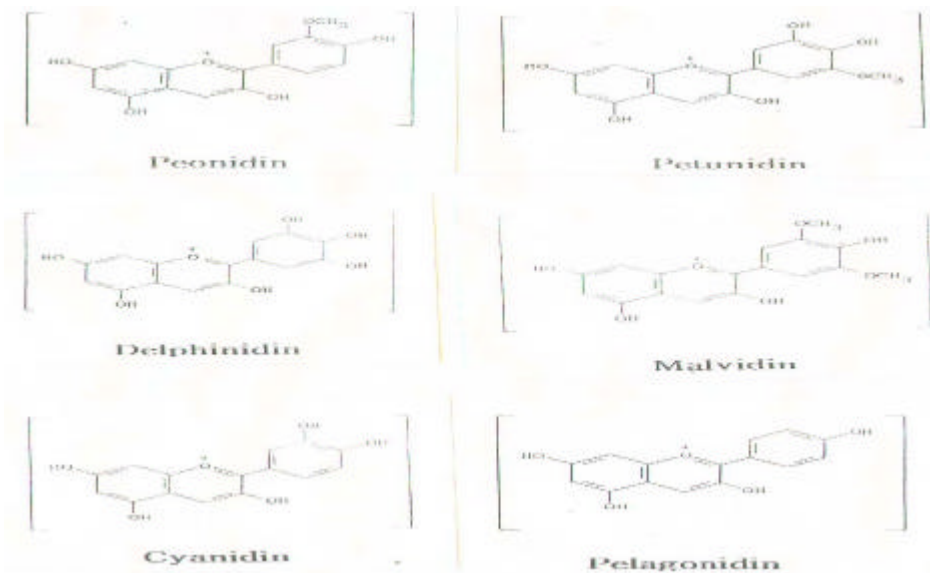
2)

가) , 0.01% HCl in MeOH 0.01% HCl in EtOH
 5% 3 가
 3.28. .

3.28. ,

	(nm)		가 (nm)
	0.01% HCl in MeOH	0.01% HCl in EtOH	
	533	541	0
	540	554	15

) 3.28. 533, 541nm
 가 .
 peonidin ,
 540, 554nm 가 15nm
 . 540, 554nm malvidin
 , malvidin 가 ,
 , cyanidin,
 petunidin, delphinidin 가
 delphinidin, petunidin 가 . , E_{440}/E_{MAX}
 25.4, 28.1 3-glycoside .
 peonidin-3-
 glycoside , malvidin-3-glycoside,
 petunidin-3-glycoside, delphinidin-3-glycoside 3가
 , 3가 가 .



3.18. Anthocyanin

3)

Fuleki Francis Philip

, peonidin-3- glucoside 86.54mg% ,
138.75mg% 2 .

1) Amberite IRC-50

가)

(Amberite IRC-50)

) charge

0.3% HCl in EtOH , 가

가

0.3% HCl in EtOH

) ,

, 가 , , ,

2)

가) 0.3% HCl in EtOH

3.7. PC , n-BuOH/

pyridine/ (6:3:1, v/v) 가 가 .

) n-BuOH/pyridine/ (6:3:1, v/v) Whatman No. 1

, 0.3% HCl in EtOH spotting

, 가 , 0.3% HCl in EtOH

가

.

1)

가) 6가 (3.18.) aglycone (anthocyanin)

anthocyanin

anthocyanin

) Anthocyanin

가

가

, Amberite IRC-50

anthocyanin

HPLC

, glucose

glucose

, 가 , , ,

2) PC

가) , , 가 , , ,

anthocyanin Whatman No. 1 spotting 3.8.

n-BuOH/ / (4:1:5, v/v, , BAW), / / (82:15:3, v/v,

WHA) 4-29.

) , 2 , 2 anthocyanin

anthocyanin

anthocyanin

anthocyanin

anthocyanin delphinidin, malvidin, petunidin

anthocyanin peonidin, cyanidin

3.29. anthocyanin PC .

							가
BAW	<i>Rf</i> 0.140	<i>Rf</i> 0.140	<i>Rf</i> 0.133	<i>Rf</i> 0.273	<i>Rf</i> 0.193	<i>Rf</i> 0.140	-
	<i>Rf</i> 0.233	<i>Rf</i> 0.273			<i>Rf</i> 0.433	<i>Rf</i> 0.273	
WHA	<i>Rf</i> 0.066	<i>Rf</i> 0.120	<i>Rf</i> 0.080	<i>Rf</i> 0.066	<i>Rf</i> 0.133	<i>Rf</i> 0.147	<i>Rf</i> 0.167
	<i>Rf</i> 0.266	<i>Rf</i> 0.273	<i>Rf</i> 0.253	<i>Rf</i> 0.233	<i>Rf</i> 0.353	<i>Rf</i> 0.273	<i>Rf</i> 0.320
			<i>Rf</i> 0.413	<i>Rf</i> 0.366			<i>Rf</i> 0.507

) anthocyanin BAW 2
 , WHA
 , 3.29. WHA가
 WHA .

) Anthocyanin

3.29. , 2 anthocyanin

Whatman No. 3MM(20 X 20cm)

anthocyanin banding WHA

, *Rf* 0.080 band, *Rf* 0.266 band가

, *Rf* 0.060 , *Rf* 0.130 band가

. *Rf* 1% HCl in MeOH .

.

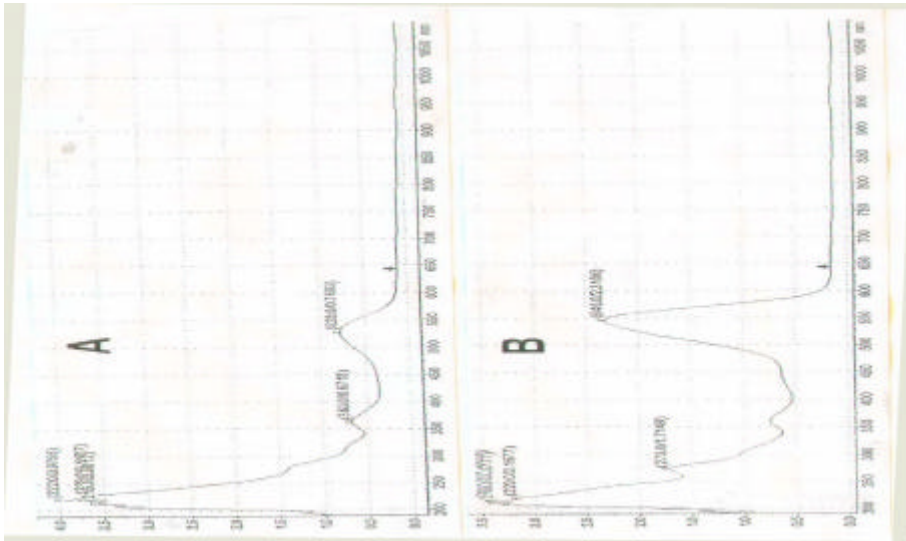
1)

가) *Rf* 0.080(-0.080), *Rf* 0.266(-0.266),

Rf 0.060(-0.060), *Rf* 0.130(-0.130)

, -0.080 535nm -0.266 528nm(3.19.) -0.060

546nm(3.19.) -0.130 538nm .



3.19. -0.266(A) -0.060(B)

) -0.080 cyanidin peonidin
 , -0.266 peonidin , -0.060 delphinidin , -0.130 malvidin

2) , PC

2 glucose
 peonidin-3- monoglucoside peonidin-3,5- diglucoside ,
 PC 2 glucose
 delphinidin-3- monoglucoside malvidin-3- monoglucoside

3) HPLC

가) 4 4-9. HPLC , -0.080
 Rt 4.032, Rt 13.042 , -0.266 Rt 4.432, Rt 12.037 , -0.060 Rt
 3.202, Rt 21.338 , -0.130 Rt 4.673, Rt 24.093 peak가 ,
 , -0.080 Rt

13.042 peak Rt 13.095 , -0.266 Rt 12.037
 peak Rt 12.282 , -0.060 Rt 21.338 peak
 Rt 20.995 , -0.130 Rt 4.673 peak Rt 4.410
 가 , 가
 .

4. : Schiznadrin

가.
 , ether MeOH .
 Ether
 () ether (ether) . MeOH
 , ethylacetate celite
 charge n-hexane, chloroform, methanol .
 , ether , n-hexane , chloroform ,
 methanol schizandrin benzene/ acetone (9:1, v/v)
 n-hexane/acetone(7:3, v/v) TLC
 , ether n-hexane
 가 chloroform 가,
 가 ether
 n-hexane , .

- 1)
- 가) Silica gel n-hexane charge
- 3.10. . 12
- schizandrin TLC (3.20.,
- 3.21.), G, H 가
- , I, J . G, H, I, J

) Silica gel n-hexane

charge

3.11.

14

schizandrin TLC

(

3.22.,

3.23.), ,

가

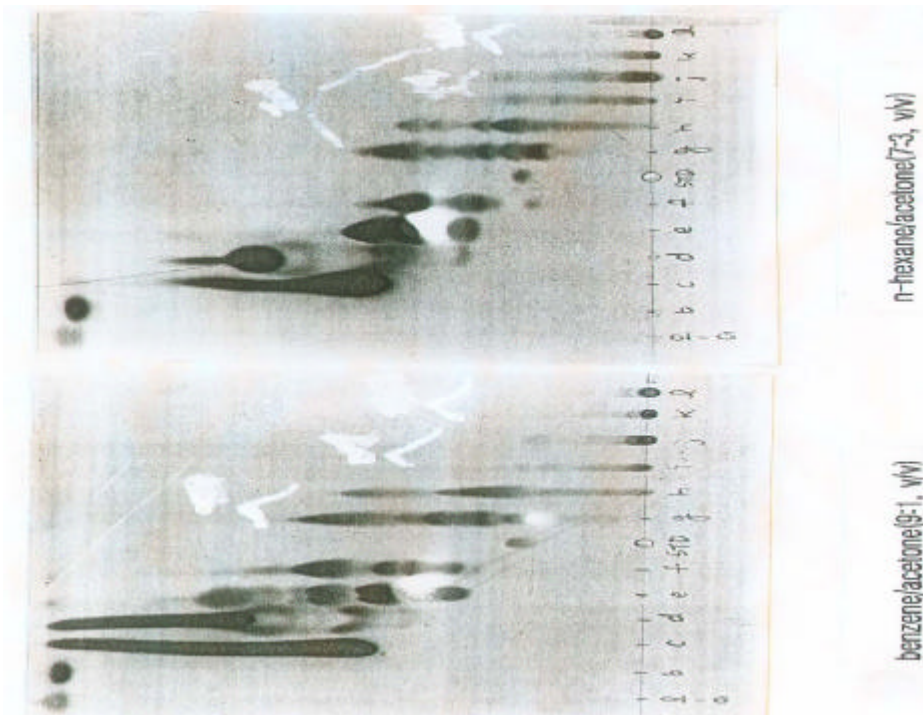
Sephadex

LH-20

2) Sephadex LH-20

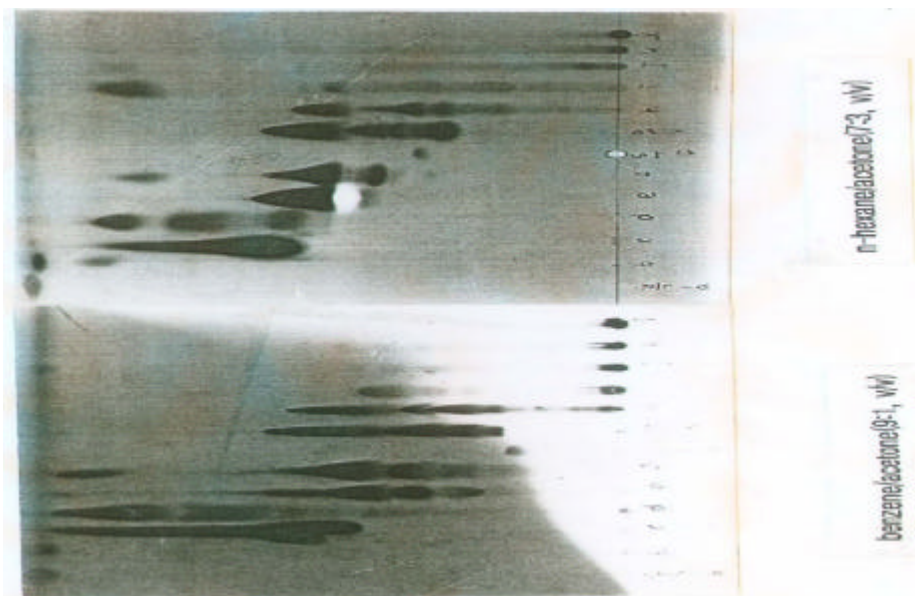
가)

Sephadex LH-20



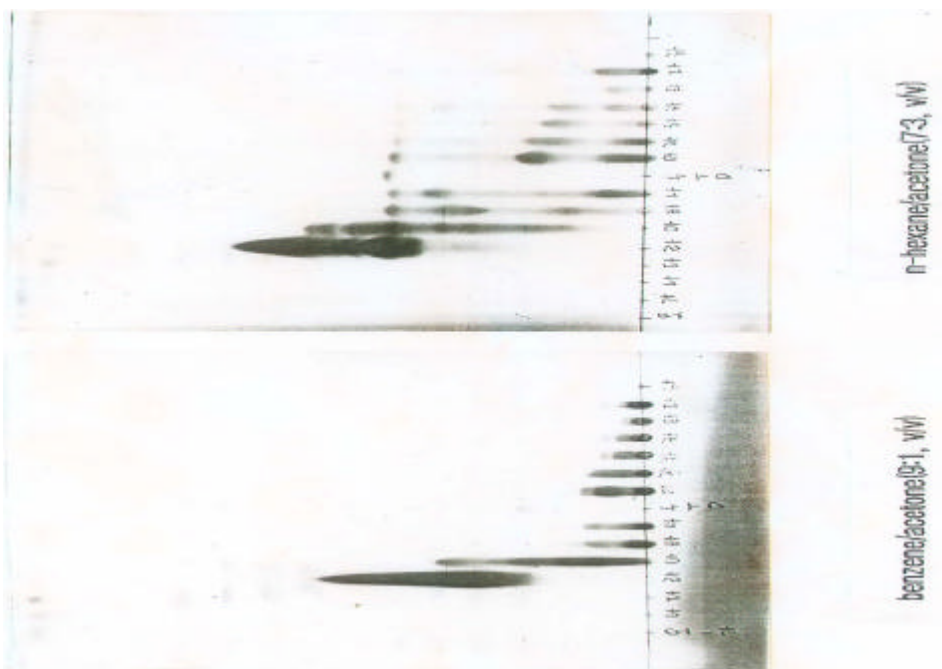
3.20.

TLC



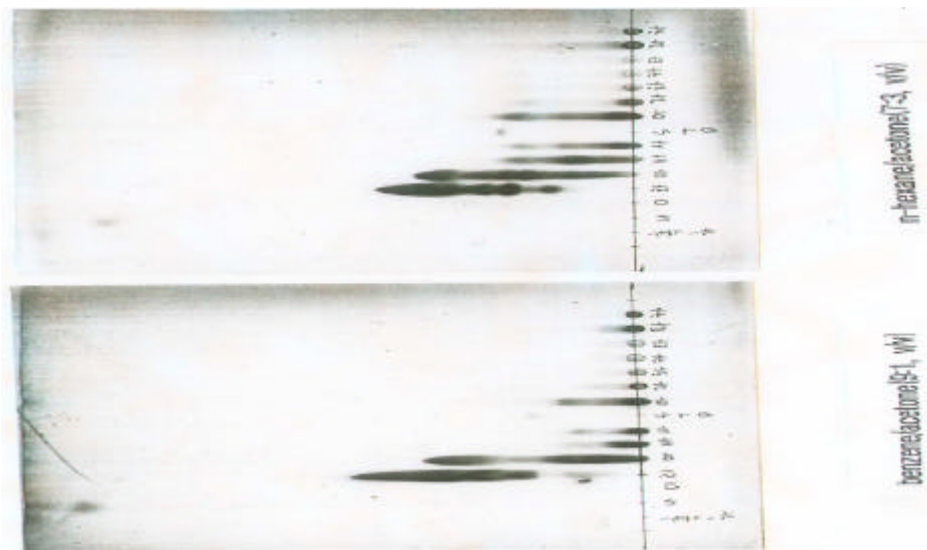
3.21.

TLC



3.22.

TLC



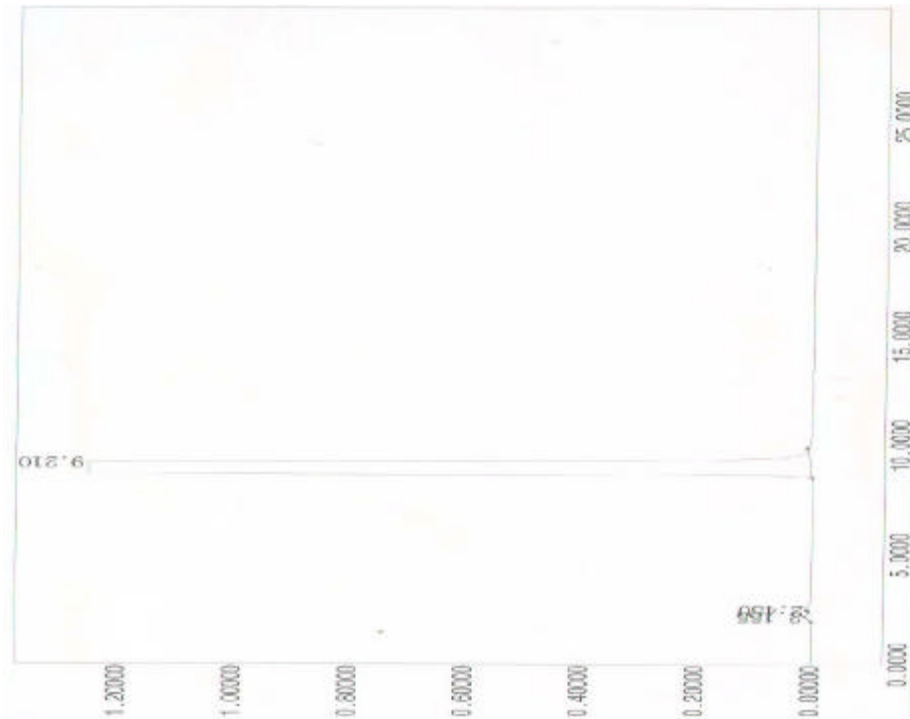
3.23. TLC

) Sephadex LH-20 chloroform/MeOH(3:1, v/v) slurry
 80ml 10 10
 schizandrin TLC , 3, 4, 5 (
 0.6 1.0) 가 . ,
 가
 3)
 가) Sephadex LH-20 가 3,
 4, 5 , TLC banding
 n-hexane/acetone(7:3, v/v) schizandrin
 schizandrin *Rf* MeOH 4 ,

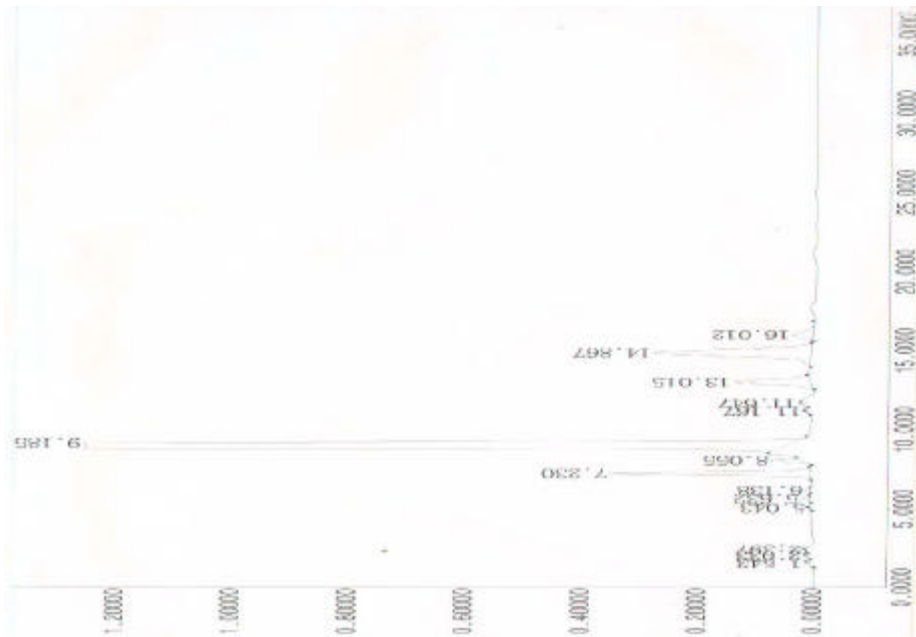
) TLC benzene/ acetone (9:1,
v/v) 가) , , , ,

. HPLC

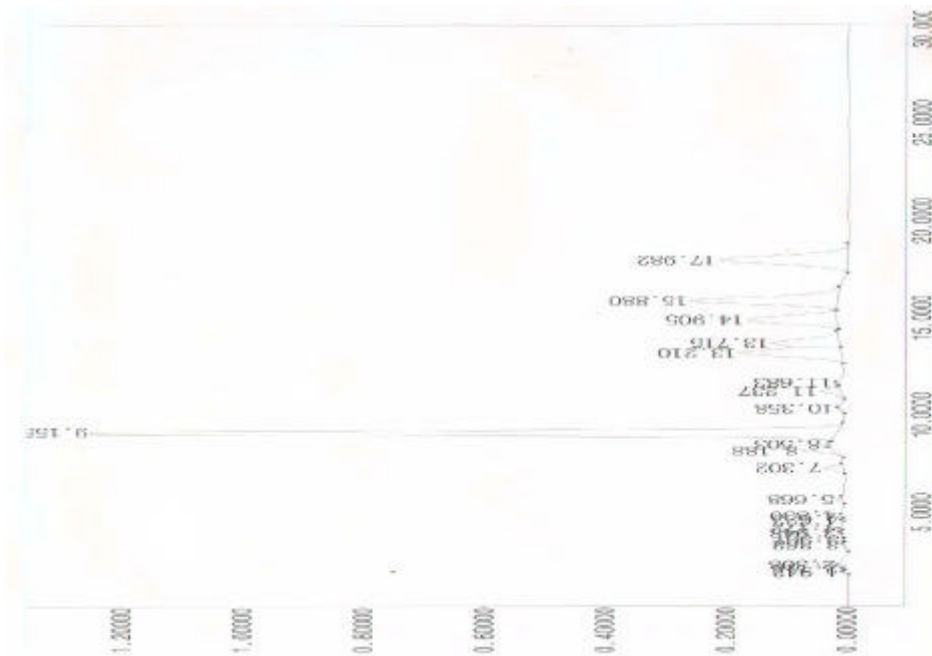
1) TLC membrane filter(Millipore, 0.45µm, Waters
) 3.12 HPLC , schizandrin
3.24, 3.25, 3.26 .
2) 3.24. schizandrin Rt 9.210 ,
3.25. Rt 9.185 peak가 ,
Rt 7.230, Rt 13.015, Rt 14.867 peak가 .
3.26. Rt 9.155 peak가 , Rt
13.210, Rt 13.715, Rt 14.905, Rt 15.880, Rt 17.982 peak가 .



3.24. Schizandrin HPLC



3.25. HPLC



3.26. HPLC

sucrose 1.13% 0.02% ,
 fructose glucose 3.71%, 2.51% 1.83%, 1.05%
 , malic acid 47,684 ppm, 38,691 ppm 가
 , citric acid 11,939 ppm, 3,330 ppm .
 malonic acid maleic acid 2.8 ppm, 2.3 ppm 0.3
 ppm, trace .
 722 mg% 7,577
 mg% , 202 mg%, 5,596 mg%
 lysine 300 mg% 288
 mg% .
 glutamic acid, aspartic acid, threonine, valine Leucine .
 1.275%, 1.560%
 , 가
 chlorogenic acid 1,276 ppm, 802 ppm , gentisic acid
 69 ppm, 136 ppm
 ferulic acid 187 ppm, 23 ppm
 . cinnamic acid, caffeic acid, coumalic
 acid .
 145 mg/g 160 mg/g
 ,
 linoleic acid가 21,765 ppm, 45,584 ppm 가 ,
 palmitic acid가 3,974 ppm, 10,872 ppm 가

capric acid .

,
 ,

-ylangene, , -elemene, -himachalene,
 -selinene widdrene ,
 caryophyllene, calarene, cubebene, acoradiene -himachalene

가 .

86.54mg% , 138.75mg%

2 .

peonidin-3-monoglucoside peonidin-3,5-diglucoside ,
 delphinidin-3-monoglucoside malvidin-3-monoglucoside

schizandrin 0.11%/100g() ,
 0.13%/100g() .

5

1

, 가

,

.

가

,

가

.

가

가가

package

2.5

.

,

가

가

, 가

가

가

가

.

가

,

가

가

.

,

,

,

가

.

, soft drink,

extract

가

()

,

,

가

.

,

가 가가 , 가
가 가
,
가 route가 . 가
가 .
가 가 가
,
UR , 가

,
, 가 가 route 가
,
,

Alloxan 가

, 茶 가
() 5~6 가 茶(
)
가
(), ()

가

가

2

1.

가. : 1996 1998 10 11 , 4

. : 1996 11

4 (± 1)

. : 1997 10

- 18 4

. : 1997 1999 1

1996 1998 가

4

. : 1997 1

0-4

. : 80 4 40 7

25%

. : 96

. :

. :

2.

가.

, , A.O.A.C. 105
 , Soxhlet , 600
 (Auto kjeldhal system, Buchi,
 Switzerland) , (Fibertec system M
 1017, Tecator, Switzerland) 100
 (가)

4가

2가

2가

4가

1) : , 20% ethanol , 25% ethanol

2) : 80 40

3) : 80 3, 4, 5 40 7

가 4

20% ethanol 5 80 3

, 4 , 5 1 1

3

45 50 30 ()

40 7

3

4

2가

2가

3, 4, 5, 7

3 (Tokyo Denshoku Co. SP-80,
 Japan) Hunter L (), a (), b ()
 spectrophotometer (UV/Visible Spectrophotometer,
 Pye Unicam, U.K.) 520nm

2ml 가 5ml 100ml 5ml Folin
 catechol 100ml 660nm

pH pH meter (Orion 720A, U. S. A)
 0.1N NaOH (pH 8.3)
 (ATAGO, Japan)

2가 (25%)

가 pH,

(4 ± 1)

60 80 4 3%

isoascorbic acid(=erythorbic acid)

0.025% 가 가 가 .
 140 150M0 Al-pack 80 5 6 17
 (4 ± 1) .
 , ,
 ,
 7
 ..
 . () , ,
 , , 3% 60 4
 5%가 가
 가 가 . Al-pack
 (4 ± 1)
 가
 .
 , , , .

3

1.

4.1.

가 86.3%

, 가
가
가 50%

가

가
가

4.1.

						(가)
()	86.30	1.05 (7.66)	2.07 (15.11)	3.21 (23.43)	0.68 (4.96)	6.69 (48.83)
()	19.17	6.23 (7.71)	16.04 (19.84)	15.22 (18.83)	3.85 (4.76)	39.49 (45.17)
()	22.12	5.58 (7.16)	13.93 (17.89)	15.21 (19.53)	3.90 (5.01)	39.26 (50.41)
()	10.41	0.84 (0.94)	1.57 (1.75)	44.11 (49.24)	2.60 (2.90)	40.47 (45.17)

() (%)

2.

가

4.2. .

4.2. (%)

(%)	80						40	
	3		4		5		7	
	0	20	0	20	0	20	0	20
()	9.32	9.68	9.52	10.08	9.40	9.16	8.31	8.40
()	45.63	39.25	47.04	39.12	47.48	45.45	38.44	35.02
()	39.78	38.56	40.79	38.74	48.61	41.53	38.39	37.63
()	12.02	12.13	13.99	15.07	14.31	13.19	9.79	9.45

(3, 4, 5) 40 (7) 80
 3
 4 가 3 80
 5
 4
 5 20%
 10% 가 80 4 가
 4 8 12%가
 , 가 1 3%
 가
 가
 (3
 5%) , 80 4
 4 80 20%

가 (,)

가

가 가

가

가

45%

90

3

가

가

3.

1)

(a)

가

(

)

가

a , b , L

a

4.3.

가

(80)

(40)

, 80

4

a

, 40

7

20%

a

가

80

5

40 7

a

a

a

4.3. (a)

(%)	80						40	
	3		4		5		7	
	0	20	0	20	0	20	0	20
()	47.57	67.62	65.97	70.05	60.81	68.62	60.07	63.44
()	26.75	27.53	28.63	27.65	27.97	29.34	32.48	35.70
()	13.38	18.36	12.58	19.14	17.39	20.60	16.55	22.16
()	3.44	11.02	5.52	10.65	7.18	12.06	0.22	2.53

(80)

a

(40

) a

20%

a

2

a

a

가

2)

(b)

가

b

4.4.

4.4.

(b)

(%)	80						40	
	3		4		5		7	
	0	20	0	20	0	20	0	20
()	30.26	56.64	58.92	41.04	68.41	56.14	34.68	50.87
()	32.33	32.69	31.83	32.90	31.87	48.04	22.46	19.02
()	21.91	32.23	23.68	36.00	18.14	40.48	14.30	19.80
()	40.91	55.08	47.59	55.90	53.40	59.62	35.19	54.24

b 가 (80 ,)

80 20%

40 7

a

가

b

5

b

a

b

5

20%

b

b
20% b

b

4.

1) (L)

, 가
L 4.5.

4.5. (L)

(%)	80						40	
	3		4		5		7	
	0	20	0	20	0	20	0	20
()	29.59	17.08	18.40	8.03	19.71	16.79	14.35	23.32
()	49.54	39.49	50.51	47.95	45.81	30.10	66.02	64.68
()	71.58	65.08	68.19	45.13	63.85	50.35	76.92	69.66
()	79.64	60.61	75.17	61.82	72.85	59.37	84.00	81.08

L () 80 4

20%

L ()

L

L ()

20%

40

7

L

L

가

가

L

alcohol

L

2)

(OD)

4가

()

520nm

4.6.

4.6.

(OD)

()	80						40	
	3		4		5		7	
	0	20	0	20	0	20	0	20
()	1.71	4.54	4.52	7.52	3.90	4.60	4.52	2.70
()	1.26	1.19	1.32	1.34	1.43	1.81	0.93	0.94
()	0.52	0.72	0.55	1.04	0.68	0.90	0.46	0.54
()	0.26	0.52	0.36	0.57	0.43	0.66	0.19	0.25

OD 80 20% 4
 3 7
 20% OD
 가
 가 20%
 가
 OD 가
 3
 2 가 ,
 2 가

5.

1)

pH
 pH 4.7
 pH 3.39 3.62
 20%
 pH
 pH 40 80
 pH가

20%

pH

pH

4.7.

pH

(%)	80						40	
	3		4		5		7	
	0	20	0	20	0	20	0	20
()	3.42	3.62	3.41	3.48	3.39	3.57	3.45	3.57
()	3.32	3.49	3.26	3.44	3.13	3.46	3.26	3.41
()	3.02	3.21	3.02	3.22	2.98	3.24	3.00	3.24
()	5.36	5.63	5.32	5.54	5.29	5.56	5.49	5.55

pH

가 가

pH

pH

가

pH 4.0

pH 4.5

, pH 5.0

pH 3.6

pH

pH 5.3

2)

4.8.

4.8.

(%)

	80						40	
	3		4		5		7	
	0	20	0	20	0	20	0	20
(%)								
()	2.2	2.6	2.6	2.6	2.4	2.0	1.4	2.2
()	2.8	2.0	3.0	2.2	2.8	2.2	2.2	2.0
()	1.6	2.0	1.8	2.0	2.2	2.0	1.6	2.0
()	0.8	0.8	1.0	1.0	1.0	1.0	0.6	0.4

80

4

4

20%

가

가 가 , ,
가

2 3%

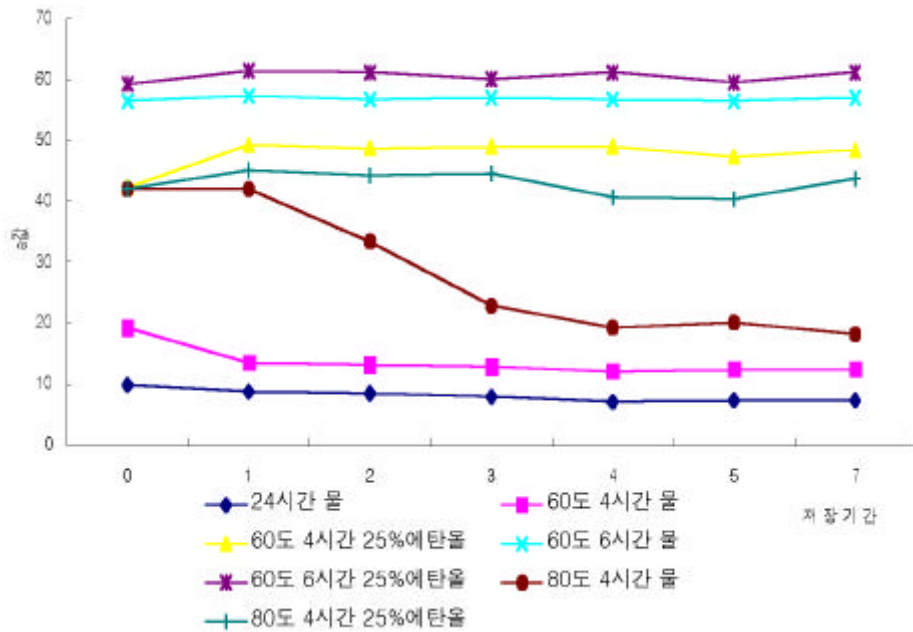
50%

3%

6.

1)

(a)



4.1.

(a)

가

Hunter a () 4.1.

1 80 4
60 6
25%

4 60 4 80
25%

(17 19) 24

가 60 6 (25%) 6

(a)

7 가

a 80 4 a 가
80 4 a 가
가

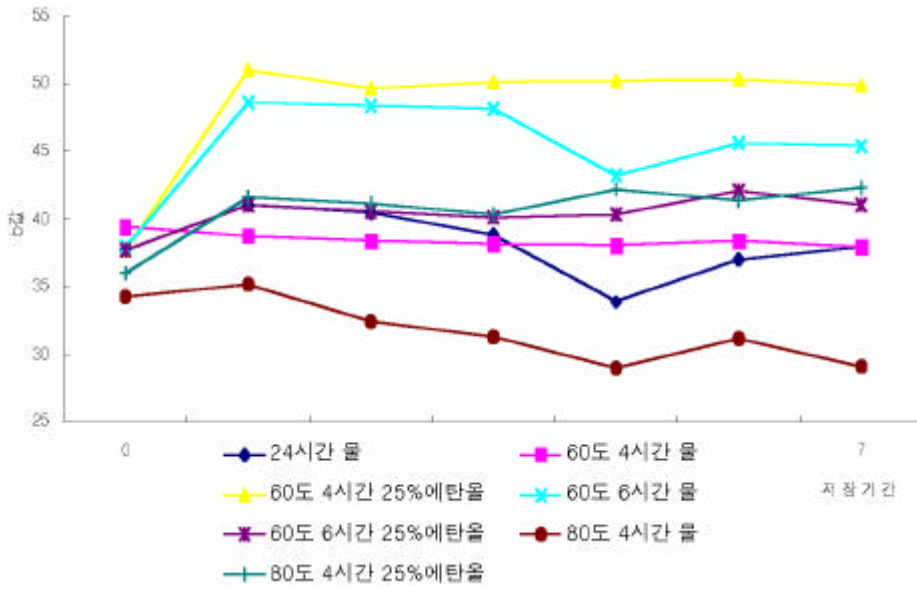
2) (b)

가 가

가

Hunter 4.2.

b () 60 4
 60 4 25% 가 가
 60 6 가 4
 가 가 80 4
 가
 24
 1 가 가 가



4.2. (b)

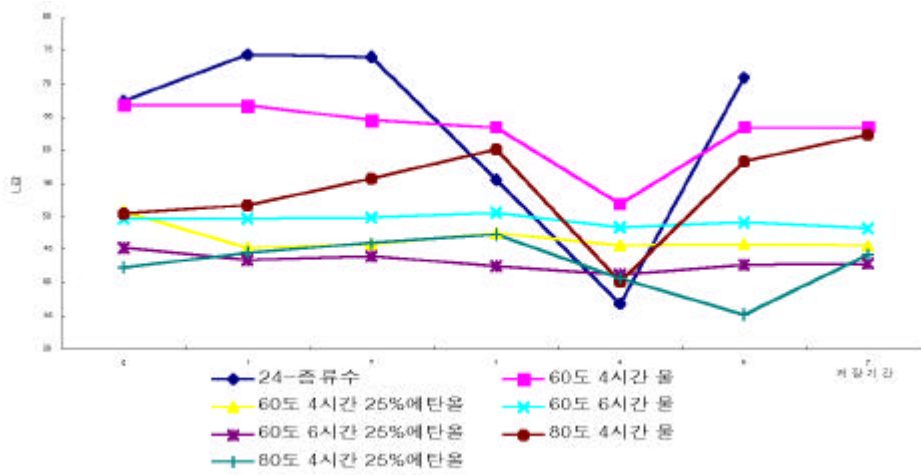
가 가 가 가 b 가
 . 80 4 , 60 4 , 60 6

a (L) 가

3)

(L)

L 4.3. . 4.3.
 L 가 24
 가 가
 가 60 4
 . 60 6 80 4
 L
 . L
 80 4 4
 4
 가 가 가
 가



4.3.

(L)

4)

520

nm

4.4.

(OD) 60 6 25%

가 가 , 60 6

24

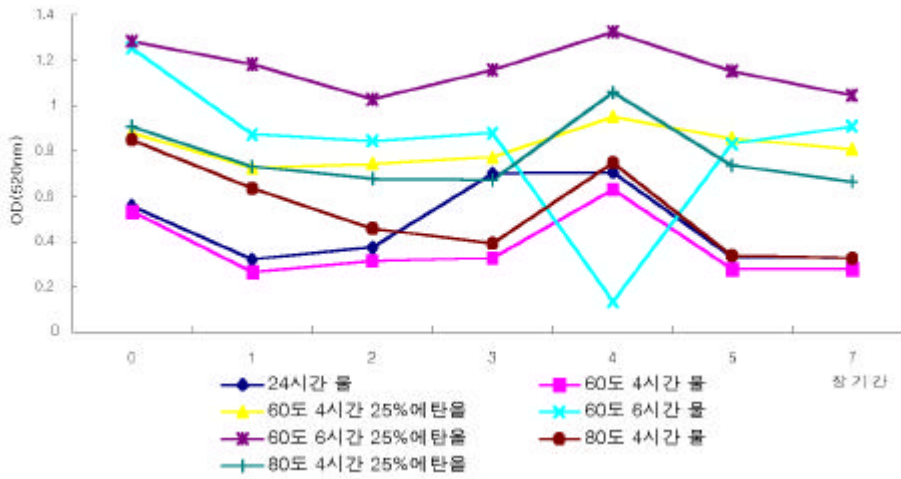
60 4

2가

4

가

3



4.4.

(OD)

5)

(total polyphenol)

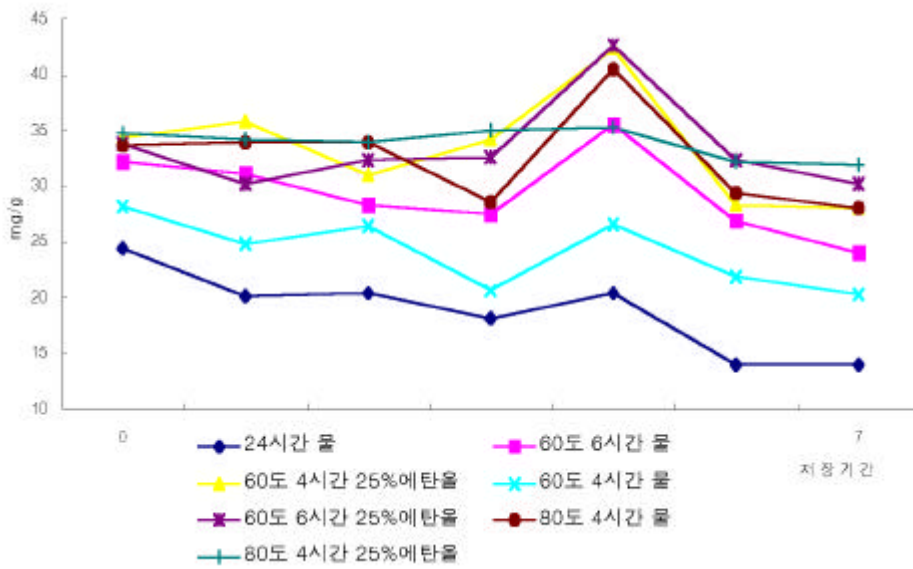
(polyphenol)

2

가

가

4.5.



4.5.

24

60 4

25%

4

4

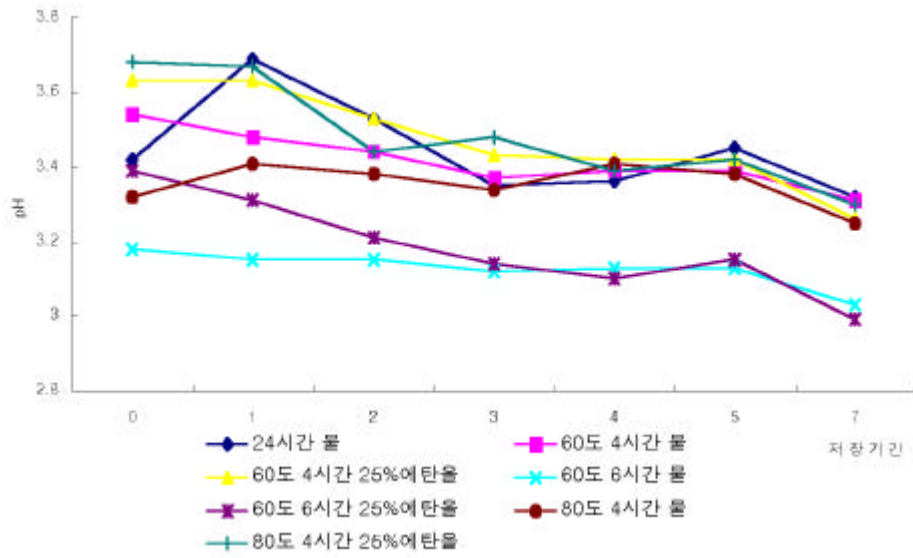
가

가 가 . 25%

가

가 가 .

6) pH



4.6. pH

pH

pH 4.6.

pH 3.68 2.99

6

0 6 25%

pH 3.6 3.3

24

pH

pH

pH

pH

pH

pH 4.0

7)

가

가

0.1N - NaOH

가

4.7.

가

60 6

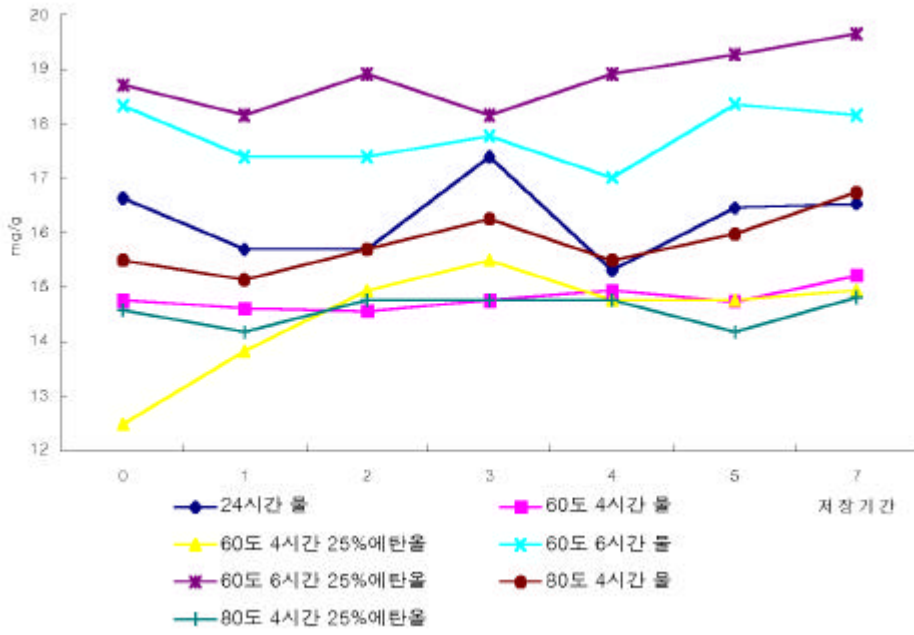
25%

가 가

24

80 4

pH



4.7.

가

가

8)

4.8.

가

가

가

3

a

4.9.

4.9.

a

	1	2	3	4	5	6	7	8	9	10	13	14	20	21
80	52.6	51.6	52.0	51.4	51.8	51.6	53.1	51.8	50.9	51.2	51.8	51.5		
	49.3	48.2	49.0	48.5	49.3	48.4	50.2	48.7	48.8	48.6	48.8	48.3		
	46.1	45.4	45.7	45.6	46.3	45.6	48.4	45.7	46.2	45.8	46.5	46.4	45.6	46.8
60	42.0	40.5	40.9	40.8	41.3	40.9	42.9	40.3	41.3	41.0	41.8	41.5	40.9	41.8
	40.2	39.8	39.4	39.3	39.3	39.6	40.2	39.4	40.1	39.4	39.2	39.2	38.9	40.9
80	36.3	35.8	35.4	34.9	36.3	35.8	37.0	35.7	35.7	35.1	34.3	35.0	34.9	33.4
	41.8	41.4	41.3	41.2	41.8	41.8	42.5	41.6	42.0	41.6	41.7	41.2	42.9	42.8
60	43.5	43.1	43.2	42.7	43.2	43.4	44.4	43.5	43.5	43.1	43.4	43.2	41.0	45.2
	11.8	11.8	11.9	11.9	7.0	8.2	8.7	7.8	8.2	7.9	8.3	8.2	6.1	5.9
60	12.2	12.0	12.1	12.0	6.8	7.8	8.3	7.8	7.9	7.8	8.0	7.6	5.7	5.5

,

,

,

60 80

1%

3%

a

가 가 가

가

가

1/4 1/5

가

4

80 가 60

가

,

가

60 가

60

3%

가

21

a ()

가

60

3%

20 (5)

가

2)

가

(a)

,

erythorbic acid

(iso ascorbic acid)

0.025%

가

가

ion

,

가

가

a

4.10.

4.10.

가

a

	1	2	3	4	5	6	7	8	9	10	13	14	20
80	50.9	50.8	51.2	51.7	51.8	50.9	51.0	52.7	53.9	50.8	51.2	50.1	
	47.2	47.4	47.4	48.0	48.0	46.6	46.2	47.7	48.4	45.9	46.1	45.8	
60	44.9	44.3	45.0	43.8	44.5	43.8	43.0	45.0	45.3	43.5	44.1	42.7	38.8
	38.3	37.3	37.6	37.8	37.8	36.9	36.7	38.2	38.0	36.3	36.2	35.4	35.4
80	36.2	36.2	36.4	36.5	36.6	35.5	35.0	36.6	37.2	35.2	32.6	34.5	35.2
	32.3	32.8	32.6	32.8	32.8	31.0	31.7	32.0	31.8	32.0	30.8	30.8	27.5
60	40.2	38.0	38.3	38.9	38.6	38.5	37.8	37.4	39.4	38.2	32.9	37.5	37.8
	42.6	40.5	40.4	40.6	39.2	40.5	39.9	40.0	41.2	39.5	39.4	39.0	39.4
60	11.8	10.0	9.8	10.5	6.8	6.6	5.8	6.8	5.8	5.8	6.2	5.7	6.4
	11.4	10.1	10.0	10.0	6.2	6.5	5.9	6.2	6.0	5.9	5.8	5.6	5.6

가

a

가

a

2 5

a

a

가

a

20 (5)

pH

3)

(b)

b

4.11.

b 80
60

가

가

3%

b

(a)

b 3 4

가

b 10

가

가

가

b 가

b

가

b

b a L

가

가

4.11.

b

	1	2	3	4	5	6	7	8	9	10	13	14	20	21
80	40.7	41.2	41.3	41.9	41.7	41.8	42.8	42.4	41.6	42.6	43.0	44.2		
	37.7	38.0	38.2	38.8	38.8	38.4	38.6	38.9	39.2	39.3	39.3	39.7		
	30.6	30.7	30.8	31.2	31.1	31.0	31.3	31.4	31.6	31.5	31.1	31.5	31.4	33.0
60	29.8	29.7	29.9	30.3	30.3	30.3	30.4	29.3	30.7	30.7	30.5	30.8	30.8	32.2
	40.7	41.0	41.7	42.3	42.9	42.7	43.4	44.0	43.0	44.4	47.0	47.3	48.2	52.4
80	30.6	30.6	30.6	30.8	31.6	31.1	31.6	31.9	32.5	32.6	32.0	33.8	34.8	39.3
	37.8	38.8	39.6	39.4	39.5	39.4	39.5	39.9	40.7	40.3	40.5	41.5	46.5	51.9
60	42.2	43.4	43.4	44.0	44.1	44.5	44.1	44.0	44.7	44.9	44.9	45.1	45.0	56.5
	10.3	10.9	10.9	11.2	6.7	7.6	7.5	7.5	9.4	7.7	8.0	8.1	6.7	6.8
60	9.9	10.4	10.4	10.7	6.4	7.2	7.4	7.3	9.5	7.6	7.6	6.7	6.4	6.3

4) 가 (b)
 , erythorbic
 acid(iso ascorbic acid) 가 , b
 4.12. .
 가 가
 . ,
 가
 .
 a erythorbic acid 가 a, b

가 .

4.12.

가

b

	1	2	3	4	5	6	7	8	9	10	13	14	20
80	39.3	38.8	40.4	41.0	42.0	40.2	42.1	42.6	42.4	42.1	42.2	41.9	
	37.0	37.2	37.8	38.5	38.9	36.2	36.8	37.2	38.3	38.5	37.6		
60	30.4	29.5	28.5	29.4	29.8	29.1	29.5	29.8	29.3	29.2	28.9	28.8	30.1
	30.3	30.0	30.4	30.6	30.7	30.4	31.0	31.1	30.3	30.7	30.1	30.4	30.0
80	38.1	34.6	36.0	35.8	35.8	36.9	39.0	39.6	37.1	36.4	33.4	38.2	37.8
	29.0	27.9	28.6	28.9	28.4	29.3	30.3	30.2	31.0	28.6	30.7	30.0	29.0
60	30.0	31.0	32.2	32.7	32.2	32.0	32.7	32.5	32.4	32.5	32.2	31.6	31.4
	35.0	36.5	37.0	36.6	36.2	36.3	36.6	36.2	35.8	35.4	34.6	35.7	34.6
60	9.6	9.4	9.8	10.2	7.2	6.4	7.2	7.4	7.3	6.9	7.4	7.1	7.6
	9.4	9.7	9.6	10.1	6.9	6.5	6.9	7.3	7.0	7.2	7.5	7.6	7.6

5)

(L)

L ()

2가

가 .

L

L

a ()

L

80 4

,

60 4

,

80 60

60 4

L

a

3%

L

가

L () 가
 , , L
 L
 3.10. a a 가 L
 , b L 가
 L 가
 가
 가 가

4.14. 가 L

	1	2	3	4	5	6	7	8	9	10	13	14	20
80	45.2	45.0	44.5	45.1	43.9	41.4	43.2	42.8	40.2	43.2	41.6	40.4	
	47.9	47.2	47.0	47.5	46.9	45.1	46.9	46.2	44.1	46.4	45.8	46.1	
60	51.5	53.2	52.1	52.8	52.4	53.3	53.5	51.0	50.7	52.7	51.3	52.5	51.6
	54.6	55.2	56.0	56.9	55.4	55.8	56.4	53.7	53.8	55.8	55.4	55.9	55.4
80	54.6	56.8	55.1	55.6	54.9	52.8	53.4	51.0	53.6	53.9	53.4	50.5	52.4
	62.9	64.5	63.6	63.6	63.4	61.5	60.6	60.9	60.9	61.3	58.3	61.5	71.9
60	60.8	62.6	61.1	61.1	60.5	59.8	59.9	58.1	57.9	59.9	57.4	59.6	59.8
	60.5	61.9	61.1	62.0	61.3	60.9	62.8	62.0	60.8	62.4	61.8	60.9	62.6
60	68.9	66.8	66.9	70.9	73.7	75.2	78.0	79.1	78.4	79.1	76.6	79.9	79.3
	79.6	79.1	79.5	79.9	80.5	80.7	85.4	84.8	84.4	85.9	84.9	84.6	85.9

7)

(OD)

520nm

4.15.

가

60

가 가

80

80

60

a

가

520nm

4.15.

	1	2	3	4	5	6	7	8	9	10	13	14	20	21
80	1.62	1.66	1.71	1.67	1.67	1.61	1.70	1.67	1.60	1.57	1.58	1.66	1.65	
	1.56	1.49	1.54	1.52	1.34	1.43	1.39	1.55	1.43	1.45	1.45	1.48	1.48	
60	1.32	1.30	1.33	1.31	1.15	1.22	1.26	1.30	1.30	1.21	1.18	1.28	1.16	1.23
	1.13	1.12	1.12	1.09	1.13	1.07	1.10	1.16	1.06	1.08	1.11	1.09	1.03	1.05
80	1.69	1.70	1.75	1.70	2.02	1.67	1.76	1.79	1.56	1.69	1.72	1.52	1.50	1.53
	1.25	1.26	1.26	1.28	1.09	1.18	1.23	1.30	1.26	1.21	1.30	1.16	1.29	1.18
60	1.77	1.62	1.66	1.70	1.49	1.54	1.62	1.63	1.62	1.70	1.62	1.55	1.52	1.54
	1.82	1.74	1.90	1.87	1.86	1.75	1.86	1.87	1.83	1.91	1.78	1.66	1.63	1.66
60	0.48	0.44	0.46	0.44	0.21	0.24	0.24	0.24	0.22	0.24	0.24	0.24	0.17	0.18
	0.38	0.37	0.38	0.24	0.18	0.19	0.20	0.20	0.19	0.19	0.20	0.23	0.14	0.14

a

가

20 , 21

가

8) 가 (OD)

가

4.16.

4.16.

가

	1	2	3	4	5	6	7	8	9	10	13	14	20
80	1.66	1.51	1.51	1.54	1.58	1.52	1.58	1.56	1.55	1.56	1.58	1.59	
	1.36	1.40	1.39	1.39	1.46	1.42	1.40	1.36	1.40	1.33	1.40	1.40	
60	1.25	1.20	1.22	1.24	1.28	1.21	1.24	1.16	1.18	1.12	1.19	1.18	1.29
	1.03	1.07	1.02	1.00	1.06	1.02	1.02	1.06	1.02	0.98	1.03	0.94	1.05
80	1.62	1.52	1.46	1.49	1.52	1.45	1.53	1.48	1.60	1.38	1.32	1.27	1.27
	1.16	1.16	1.10	1.10	1.15	1.09	1.18	1.12	1.13	1.07	1.08	0.97	1.00
60	1.62	1.49	1.46	1.49	1.51	1.43	1.49	1.38	1.40	1.34	1.37	1.32	1.28
	1.74	1.66	1.64	1.61	1.65	1.57	1.69	1.49	1.55	1.38	1.42	1.33	1.32
60	0.43	0.44	0.42	0.41	0.23	0.23	0.22	0.23	0.20	0.20	0.22	0.20	0.18
	0.34	0.34	0.32	0.32	0.18	0.22	0.17	0.17	0.17	0.16	0.16	0.17	0.17

60 4 80 가 가 가 가
가 가 . 20

가 ,
9) 가 ,
가

(polyphenol) 가

4.17. , ,
polyphenol

가 가 가 가 가 가
가 가 가 가 polyphenol

polyphenol 14 21 20

가 가 가 가 가 가
polyphenol 가 가

4.17.

(mg/100M_l)

	1	2	3	4	5	6	7	8	9	10	13	14	20	21
80	72.8	73.5	74.2	73.5	67.0	67.6	72.5	63.8	70.4	68.2	67.8	67.1	64.9	
	72.8	72.1	73.5	72.1	60.0	71.1	71.1	67.1	68.5	66.7	66.7	68.5		
60	67.3	68.0	69.3	67.3	65.0	64.8	66.7	60.5	60.2	64.8	62.3	63.4	61.2	48.4
	67.3	66.6	68.0	67.3	64.0	59.1	66.2	65.1	64.8	64.8	64.4	58.0	57.7	48.4
80	57.7	56.6	59.8	58.7	55.2	55.3	56.4	54.6	56.3	56.9	54.4	54.9	54.9	43.6
	57.1	55.5	57.7	57.1	50.4	51.8	55.8	52.4	54.7	55.0	49.5	53.6	49.5	45.3
60	57.1	56.6	58.2	57.1	54.4	53.8	56.8	52.7	54.6	55.5	53.8	53.0	55.5	43.6
	56.0	56.0	58.2	57.1	53.6	54.6	56.8	52.4	53.8	55.3	52.7	53.5	52.7	43.6
60	66.0	67.6	67.6	68.0	59.6	61.8	61.2	59.0	58.4	58.1	59.1	56.8	55.2	43.2
	64.0	68.4	66.0	64.4	58.4	56.1	60.0	59.6	58.1	57.8	57.8	57.1	56.4	41.2

10) 가

erythorbic acid 가

4.18.

가 가

1

가

가

가

20

가

14

가

가가 가

4.18.

가

pholyphenol (mg/ 100Mℓ)

	1	2	3	4	5	6	7	8	9	10	13	14	20
80	74.9	72.8	73.9	70.0	72.4	73.4	71.6	69.4	70.7	70.5	72.6	69.8	
	73.5	72.1	72.5	70.7	72.1	73.3	71.3	67.0	70.4	69.5	72.6	68.6	
60	71.4	68.7	69.4	67.3	65.3	71.6	75.9	66.6	66.2	67.3	68.0	61.9	64.8
	70.0	68.7	69.4	68.7	69.4	72.0	72.3	64.3	66.2	59.8	64.4	60.9	64.1
80	59.3	58.7	58.7	57.1	55.7	61.4	61.5	55.5	58.1	55.4	57.5	55.3	54.4
	58.7	56.6	57.6	55.4	53.3	60.9	61.1	53.2	55.7	54.3	55.3	53.6	51.8
60	58.7	55.7	58.2	55.5	54.1	60.8	59.6	56.0	56.4	54.4	56.9	54.7	54.7
	58.2	58.2	57.6	55.5	54.3	62.6	60.7	56.0	55.5	53.5	56.3	55.0	54.1
60	69.2	67.6	69.6	67.8	67.8	67.9	69.2	66.8	63.3	65.5	61.5	60.1	59.8
	67.6	66.8	66.0	67.2	66.6	66.6	68.4	65.1	60.6	64.5	60.5	59.9	60.1

11)

4.19.

가

1/3

60 80

4.19.

(%)

	1	2	3	4	5	6	7	8	9	10	13	14	20	21
80	0.8	0.8	1.0	0.9	1.0	1.1	1.1	1.4	1.0	1.1	1.4	1.2		
	0.8	0.6	0.6	0.9	1.0	1.0	1.0	1.2	1.0	1.0	1.2	1.0		
60	1.0	0.8	1.0	1.1	1.2	1.2	1.2	1.4	1.4	1.4	1.6	1.2	1.8	1.8
	0.8	0.6	0.8	0.9	1.0	1.0	1.1	1.2	1.1	1.1	1.4	1.2	1.4	1.4
	1.6	1.6	1.8	1.7	1.9	1.9	1.9	1.9	1.9	2.1	2.1	2.1	2.1	1.9
80	1.1	1.0	1.1	1.4	1.3	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.7	1.6
	1.0	1.4	1.6	1.6	1.6	1.6	1.7	1.8	1.8	1.8	1.6	1.8	1.8	1.9
60	1.4	1.4	1.6	1.6	1.6	1.6	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.9
	0.6	0.6	0.7	0.7	0.4	0.4	0.5	0.6	0.4	0.5	0.6	0.5	0.5	0.4
60	0.5	0.5	0.6	0.7	0.3	0.3	0.4	0.4	0.4	0.4	0.5	0.4	0.4	0.4

가

가

가

가

가 가

12) 가

가

4.20.

4.20.

가

(%)

	1	2	3	4	5	6	7	8	9	10	13	14	20
80	0.8	0.8	0.8	1.1	1.2	1.0	1.0	1.2	1.0	1.2	1.2	1.2	
	0.8	0.8	1.0	1.0	1.0	0.8	1.0	1.2	1.0	1.0	1.0	1.4	
	1.0	1.0	1.4	1.1	1.3	1.0	1.4	1.4	1.4	1.6	1.8	1.8	2.0
60	0.8	0.8	1.2	1.0	1.2	1.0	1.0	1.2	1.0	1.2	1.4	1.4	1.4
	1.8	1.6	1.8	1.7	1.8	1.8	1.9	1.9	1.9	1.9	2.1	2.1	2.1
80	1.6	1.4	1.4	1.5	1.4	1.2	1.6	1.6	1.6	1.6	1.6	1.6	1.4
	1.6	1.4	1.6	1.6	1.8	1.6	1.8	1.9	1.8	1.9	1.9	1.9	1.9
60	1.6	1.4	1.6	1.6	1.8	1.6	1.8	1.9	1.6	1.9	1.9	1.9	1.9
	0.8	0.7	0.8	0.8	0.5	0.4	0.5	0.5	0.4	0.5	0.6	0.6	0.4
60	0.6	0.6	0.6	0.8	0.4	0.4	0.4	0.5	0.4	0.4	0.5	0.4	0.5

가

가

가 가
가
0.1%
가
가
가 1
가

13)

가
가
4.21.
가
가
3%
가
1/3 1/6
가
가
20
6
가

4.21.

(mg/g)

	1	2	3	4	5	6	7	8	9	10	13	14	20
80	42.8	43.5	43.7	43.7	42.0	41.3	38.8	41.3	40.1	38.0	39.3	41.6	
	42.2	43.5	42.8	43.7	40.9	40.4	37.7	39.4	41.1	39.1	40.5	40.6	
60	23.3	22.9	21.4	23.6	23.3	22.7	22.7	22.1	23.4	21.3	21.3	22.3	
	23.6	23.1	24.0	24.2	23.8	23.0	23.5	21.8	23.3	23.4	22.5	23.9	
80	146.1	131.4	130.6	153.8	140.2	140.2	140.0	138.2	139.5	138.0	136.6	136.3	130.2
	148.7	143.0	143.4	157.7	144.2	141.5	140.9	125.8	143.8	133.2	137.8	138.9	134.9
60	122.6	138.2	137.4	142.6	125.4	123.0	128.8	129.1	129.1	127.1	142.6	129.8	120.9
	115.8	118.6	109.6	122.7	113.6	109.0	112.9	115.5	110.4	110.0	^{1103.} ₇	109.5	107.9
60	151.5	150.6	151.8	145.7	145.6	123.5	123.2	121.8	107.0	116.2	123.6	123.6	113.5
	147.3	143.8	150.1	149.6	151.5	123.5	126.8	114.9	119.4	120.9	123.6	125.6	116.0

14) 가

가

4.22.

가 가

가 가

,
가

5

20

가

pH

가가

4.22.

가

(mg/ g)

	1	2	3	4	5	6	7	8	9	10	13	14	20
80	45.7	44.7	43.9	44.8	43.4	42.3	43.4	42.4	42.7	45.8	44.2	43.4	44.5
	45.9	39.4	43.4	45.9	44.2	42.5	43.4	45.7	46.5	41.1	42.4	42.4	44.2
	24.2	22.9	25.2	28.7	24.4	24.1	23.0	23.1	25.1	22.9	24.1	25.6	
60	24.5	25.9	27.8	27.9	25.2	25.8	21.9	22.3	27.0	26.1	24.1	24.7	
	148.5	133.7	145.4	149.4	138.6	141.3	137.8	137.7	141.2	138.6	125.0	139.5	135.3
80	146.7	141.4	147.6	159.2	145.0	143.4	144.8	139.5	139.0	138.5	115.0	143.0	141.8
	139.8	131.1	140.8	139.8	127.4	131.0	135.0	130.6	131.1	125.7	116.9	133.0	141.8
60	148.2	114.1	120.2	119.0	116.8	116.6	113.3	103.0	116.6	109.5	109.9	103.2	109.8
	148.3	136.0	150.4	153.7	124.3	125.5	121.7	121.8	123.7	123.2	114.5	127.6	128.9
60	155.4	143.2	151.6	149.8	123.3	126.6	122.5	117.9	119.2	123.6	115.2	124.6	125.6

15)

pH

,

pH

4.23

.

pH

.

16) 가 pH

(iso ascorbic acid)

가 가 pH

4.24. .

4.24. , 가 pH

	1	2	3	4	5	6	7	8	9	10	13	14	20
80	3.05	3.06	2.96	3.06	3.15	3.06	3.14	3.06	3.01	2.73	3.16	3.09	2.80
	2.96	3.01	2.96	3.10	3.07	3.05	3.12	2.97	2.47	2.77	3.13	3.20	2.80
	3.31	3.39	3.23	3.35	3.35	3.29	3.41	3.41	3.27	3.33	3.35	2.86	
60	3.29	3.30	3.23	3.31	3.33	3.26	3.38	3.30	3.24	3.20	3.32	2.82	
	2.72	2.78	2.64	2.80	2.76	2.76	2.83	2.70	2.64	2.59	2.69	2.71	2.65
80	2.68	2.70	2.57	2.52	2.71	2.70	2.88	2.69	2.64	2.53	2.59	2.67	2.54
	2.75	2.78	2.66	2.79	2.76	2.75	2.78	2.73	2.68	2.60	2.80	2.76	
60	2.65	2.68	2.61	2.51	2.69	2.74	2.83	2.73	2.66	2.66	2.79	2.72	
	2.81	2.92	2.73	2.98	2.72	2.77	2.97	2.84	2.79	2.70	2.74	2.69	
60	2.82	2.89	2.75	2.92	2.81	2.75	2.91	2.80	2.77	2.79	2.78	2.70	

가 pH 2.80 3.20 2.82

3.41 pH 2.53 2.88 2.66 2.80

pH 2.69 2.97 가 pH

가

pH가

가 가가

가

4

가

가

가 가

()

(,)

가

가

가

가

가

가

80

4

60

6

가

5 6 ,

가

pH

4

7

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