KOREAN J. DIETARY CULTURE
Vol. 9, No. 2 (1994)

Flavonoidal constituent in Korean *Lactuca dentata Makino*

Kang Hyun Chung*, Kwang Ro Yoon and Jun Pyong Kim**
**Korea Food Research Institute*
***Department of Food Science and Technology, Chung-Ang University*
(Received March 31, 1994)

한국산 씀바귀의 Flavonoid 성분에 관한 연구

정강현* · 윤광로** · 김준평
*한국식품개발연구원
**중앙대학교 식품가공학과
(1994년 3월 31일 접수)

Abstract

The ethylacetate extract of *Lactuca dentata Makino* showed 6 flavonoidal components as detected by ferric chloride solution. The flavonoidal constituent of *Lactuca dantata Makino* was isolated and purified by the series of column chromatography. The chemical structure of one of the flavonoidal component named as compound E was identified by UV, IR and NMR spectrometry. The melting point range of compound E was 249.5℃-251℃. The UV and IR spectra of purified compound E, and its genin were measured with the various shifting agents. The results of UV analysis showed the free state of hydroxy group at 3rd and 4th carbon and binding of sugar at the 7th carbon of compound. The sugar bound to the compound E was identified as glucose by TLC. The IR spectrum showed the presense of hydroxy group, conjugated carbonyl group and aromatic group. The analysis of NMR spectrum was done to the purified compound and its derivatives. The chemical shifts against hydrogen atom, hydroxy group, and the moiety of luteolin were observed in the NMR spectrum along with their position and number as well as type of sugar bound. The isolated and purified compound was identified as luteolin-7-0-β-D-glucoside.

I. Introduction

Lactuca dentata Makino is one of the traditional edible plant which is growing in the southern part of Korea and Japan in wild. It contains milky juice in leaves and stems, giving unique bitter taste. These kinds of plants have been consumed as types of salad and Kimchi(Fermented vegetable) in Korea for a long time[1,2].

Mark and Stewar[3] reported that the flavonoidal components such as anthocyanin and chalcone have been used as a natural coloring agent in food industry because they can be a good substrate for polyphenol oxidase. Kühanan[4] demonstrated that the flavonoidal components of plant leaves and fruit play an important role in preventing oxidation of vitamin C. Mark and Stewar[3] also revealed that the quercetin separated from plant have a strong anti-oxidative effect on lard and beef fat especially on unsaturated fatty acid. Mabry et al.[5] decleared that flavonoidal constituents possess the anti-inflammatory effect, anti-diarrhea effect and strengthening effect of capillary blood vessel. Woldecke and Herrman[6] isolated and identified quercetin-3-β-D-glucuronide from lettuce which is a similar species of *Lactuca dentata* Makino. The presense of apigenin-7-0-glucoside and quercetin-7-0-galactoside from *Lactuca indica* were identified by Kaneta et al[7]. Chen et al.[8] isolated the stearyl palmitate, stearyl stearate, lupenyl acetate from *Lactuca chinensis Makino*. Tozaburo et al.[9] identified the presense of luteolin, quercetin apigenin from *Uiburrnum dilatatum THUNB*, and Tashichiro et al.[10] separated the

luteolin-7 glucoside from *Lactuca repens Maximum.* However, limited imformation was available about chemical constituents of *Lactuca dentata Makino* except amono acid composition, chlorogenic acid and the changes of vitamin c during cooking[11,12]. The objectives of present research is to identify the chemical structure of flavonoidal constituent of *Lactuca dentata Makino.*

II. Material and Method

1. Sample preparation

Lactuca dentata Makino was collected from hoticultural farm in Kyunggi-Do Korea. The sample was dried and cut at the shady place. The sample was extracted with hot methanol(90%) at the water bath 3 times for every 4 hours and defatted with diethylether. The extracts was concentrated to the syrup at vacuum.

2. Extraction and isolation of flavonoidal constituents

The flavonoidal components dissolved in liquid phase were extracted with ethlyacetate several times at the separatory funnel. The combined extract was evaporated under vacuum and subjected to run thin layer chromatography. The developing solvents include EtOAc : MeCOEt : HCOOH : H_2O(5 : 3 : 3 : 1, v/v) and $CHCl_3$: CH_3OH : H_2O(6 : 4 : 1, v/v). The presense of flavonoidal compound was detected by 2% alcoholic ferric chloride. The isolation of flavonoidal component was done by the series of column chromatography according to Fig. 1. Silica gels were used as packing material for column chromatography. Purification for isolated compound was done by repeated crystalization in cold methanol.

3. Identification of isolated compound

The homogeneity of purified compound was confirmed by thin layer chromatography before determining its chemical structure through instumental analysis.

The melting point of purified compound was determined by melting point apparatus(Fisher Johns melting point apparatus, USA). The UV spectrum of compound in methanol was measured by spectrophotometer(Varian cray 219 spectrophotometer, USA) along with various shifting agents including sodium acetate, boric acid, aluminum chloride and hypochrolic acid. IR Speectrum of the sample was analyzed by IR spectrophotometer(Jasco A-3 spectrophotometer, USA). The testing sample was prepared as a form of KBr tablet.

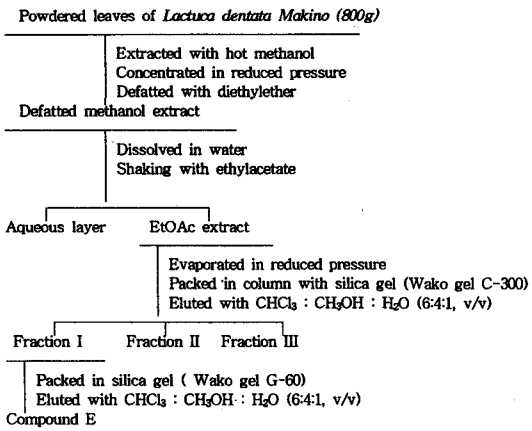

Fig. 1. Isolation of flavonoidal compound from *Lactuca dentata Makino.*

The purified compound was hydrolyzed for 4 hours in 2 N H_2SO_4 at water bath with reflux to analyze the genin(hydrolysate) and sugar part of the compound. The genin part was crystalized in cold methanol, whose homogeneity was confirmed by thin layer chromatography with different developing solvents ; benzene : pyridine : formic acid(36 : 9 : 5, v/v) and toluene : pyridine : ethylformate(5 : 4 : 1, v/v). The analysis of UV and IR spectra of the genin were also done. The aqueous portion of hydrolysate was neutralized by barium carbonate to obtain sugar potion of the compound. The sugar was identified by the comparision of Rf value of sample with that of autentic sugar sample in thin layer chromatography with different developing solvents ; ethlyacetate : pyridine : acetic acid : water(5 : 5 : 1 : 3, v/v) and n-butanol : acetic acid : ether : water(9 ; 6 : 3 : 1, v/v).

The purified compound was acetylated to analyze the characteristics of functional groups of the compound. The proton NMR specta of compound and its acetyl derivatives were measured by NMR spectrometer(Varian T-60A NMR spectrometer, USA). Tetramethlysilane was used as internal standard at 60

MHz. The solvent to solubilize the sample was DMSO-D_6 and $CDCl_3$. The chemical shift of the spectrum was expressed to ppm(δ) with spectral width of 500 Hz at 30℃.

III. Results and discussion

The ethylacetate extract of *Lactuca dentata Makino* showed 6 different spots on thin layer chromatographic system whose elution solvent were chloroform : methanol : water(6 : 4 : 1, v/v) and ethylacetate : methylethylketone : formic acid : water(5 : 3 : 3 : 1, v/v). The detection agent was 2% alcoholic ferric chloride solution. The each spot was named as A through F in order of their polarity. Compound E seemed to be main component of flavonoidal constituent of *Lactuca dentata Makino.*

The compound E was isolated by column chromatography with elution system of ethylacetate : methylethylketone : formic acid : water(5 : 3 : 3 : 1, v/v) followed by other elution system chloroform : methanol : water(6 : 4 : 1, v/v). The isolated compound E was purified by recrystalization in methanol at refrigerator. The appearance of compound E was niddle-like crystal showing light yellowish color. The purified compound E was subjected to melting point analyzer. The range of melting point of Compound E was 249.5℃-250.0℃ which was similar to that of luteolin glycoside.

In order to elucidate the chemical structure of compound E, the UV spectrum was analyzed with several shift reagent solution. Table 1 showed spectra of compound E at the various shift reagents. When sodium acetate was added to Compound E, bathochromic shift was occured at the band I(300-380 nm) and the band I was partially returned to the original specturm by the addition of boric acid which implied the free hydroxy group of 3rd and 4th position of carbon of compound E[13,14]. On the other hand, there was no chemical shift at the band II(240-280 nm) of compound E by addition of sodium acetate suggesting that the 7th hydroxy group is not free state, previewing the bind of sugar[14,15]. The compound E considered as glycoside was hydrolyzed with 2 N H_2SO_4 to find out the structural moiety of compound E and its bound sugar. The UV spectrum of genin obtained from compound E was analyzed at the same shift reagent as intact compound E . The results appeared in Table 2 showed that there was no chemical shift in band II by the addittion of sodium acetate, which indicate the free state at the 7th hydroxy group of compound E at which sugar was bound before hydrolysis. The bathochromic shift was occured in band I by addition of alumium chloride and the hypsochromic shift was happened by the addition of hypochloric acid[15,16]. These phenomena indicate the free state of hydroxy group at the position of 3rd and 4th carbon of compound E. Results from analyzing UV spectra of compound E and its genin were able to assume compound E as a luteolin glycoside because they showed free hydroxy group at 3rd and 4th carbon and sugar-binding at hydroxy group of

Table 1. UV spectra of compound E

Shift reagent	(λ max)			
λ_{max}^{MeOH}	256	269 (sh)	340	
$\lambda_{max}^{MeOH+NaOMe}$	270	330 (sh)	398	
$\lambda_{max}^{MeOH+NaOAC}$	265	328 (sh)	378	
$\lambda_{max}^{MeOH+NaOAC+H_3PO_3}$	258	300 (sh)	400	
$\lambda_{max}^{MeOH+AlCl_3}$	274	300 (sh)	430	
$\lambda_{max}^{MeOH+AlCl_3+HCl}$	268 (sh)	276	298 (sh)	386

*sh: shoulder

Table 2. UV spectra of genin of compound E

Shift reagent	(λ max)			
λ_{max}^{MeOH}	256	26 (sh)	340	
$\lambda_{max}^{MeOH+NaOMe}$	267	298 (sh)	394	
$\lambda_{max}^{MeOH+NaOAC}$	259	269 (sh)	355 (sh)	400
$\lambda_{max}^{MeOH+NaOAC+H_3PO_3}$	260			400
$\lambda_{max}^{MeOH+AlCl_3}$	278	298 (sh)		412
$\lambda_{max}^{MeOH+AlCl_3+HCl}$	278	292 (sh)		388

*sh: shoulder

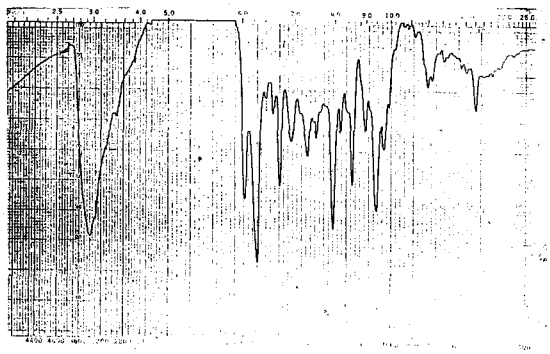

Fig. 2. IR spectrum of compound E.

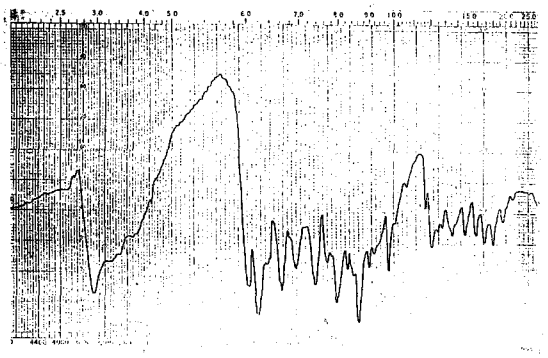

Fig. 3. IR spectrum of genin of the compound E.

7th carbon of compound E.

The IR spectrum of compound E, its genin and heptaacetate were shown in Fig. 2, 3 and 4, respectively. The presense of hydroxy group(3300 cm^{-1}), conjugated carbonyl group(1600 cm^{-1}) and aromatic group(1600, 1500, 2nd 1450 cm^{-1}) were shown in IR spectrum of compound E in Fig. 2[15,17]. The IR spectrum of genin of compound E in Fig. 3 showed similar pattern as compound E implying the presense of hydroxy group, conjugated carbonyl and aromatic groups. The IR spectrum of heptaacetate of compound E was shown in Fig. 4. The unique absorption bands due to ester carbonyl band(1750 cm^{-1} and 1240 cm^{-1}) were shown in the spectrum along with conjugated carbonly group(1650 cm^{-1}) as well as aromatic group(1750 cm^{-1})[18]. The pattern of IR spectra obtained from compound E, its genin and haptaacetate showed similar pattern as those of luteolin-7-glycoside identified by Ulube[15] and Tomimori[17].

The analysis of proton NMR spectra for compound E and its derivartives were done to assure the posi-

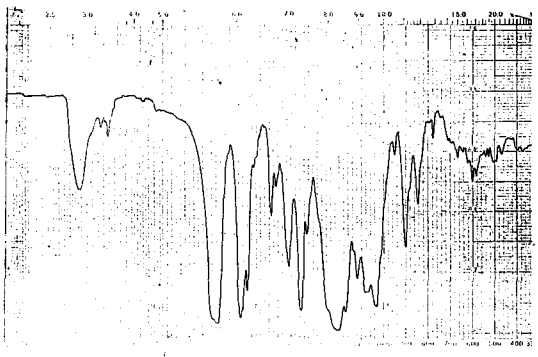

Fig. 4. IR spectrum of acetate of the compound E.

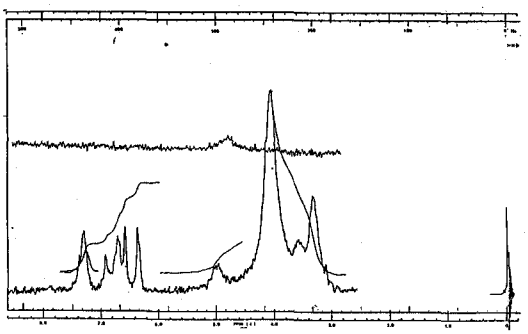

Fig. 5. NMR spectrum of the compound E.

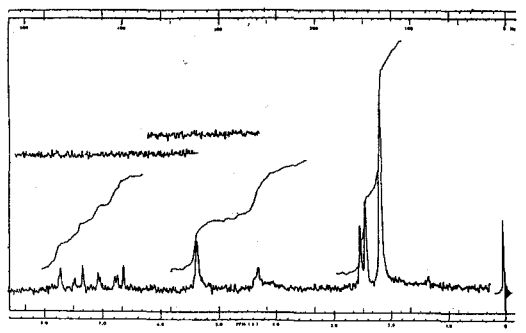

Fig. 6. NMR spectrum of acetate of the compound E.

tion of its functional component. The proton NMR spectrum of compound E shown in Fig. 5 represent six proton signal at 6.35(1 H, d, J−2 HZ, C_6-H), δ6.6(1 H, s, C_3-H), δ6.68(1 H, d, J=2 HZ, C_8-H), δ6.87(1 H, d, J=8 HZ, C_5'-H), δ7.32(1 H, d, J=2 HZ, C_2'-H), and δ7.33(1 H, d, J=8 Hz, C_5'-H). The chemical shift due to C_5-OH and anomeric proton were occured at δ12.5 and δ5.0, respectively[13,14]. The proton NMR spectrum of heptaacetate of compound E was shown

in Fig. 6. The proton signal due to acetylation of sugar was observed at δ2.2(1 H, s, 4×$COCH_3$) and the proton signal from luteolin moiety was appeared at δ2.47(1 H, s, $COCH_3$) and δ2.53(1 H, s, 2×$COCH_3$). The four proton signal due to the presense of sugar were shown at the δ5.38(3 H, s, C-2″, 3″, 4″) and δ4.25(2 H, s. C-5″). Six proton signals from luteolin moiety were occured at δ6.67(1 H, s, C_3-H), δ6.75(1 H, d, J=2 Hz, C_6-H), δ7.05(1 H, d, J=2 Hz, C_8-H), δ7.4(1 H, d, J=8 Hz, C_5'-H), δ7.75(1 H, d, J=2 Hz, C_2'-H) and δ7.8(1 H, d, J=8 Hz), C_6'-H)[13,14]. The anomeric proton signal was also obvserved at δ5.86(1 H, s, J=8 Hz). The coupling constant(J) of 8 Hz in anomeric proton signal can tell that the pattern of sugar-binding is β type[14]. The PMR spectra of compound E and its derivatives showed same as luteolin-7-0-β-D-glucoside identified by Ulubelen[15] and Kagan[16] from other plants. Therefore, it is obvious that the compound isolated from *Lactuca dentata Makino* is luteolin-7-0-β-D-glucoside.

IV. Conclusion

The ethylacetate extract of *Lactuca dentata Makino* showed 6 different flavonoidal constituents in the thin layer chromatography. One of the component named as compound E was isolated and purified by various conditions of column chromatography. The melting point of compound E was 249.5℃-251℃ The structural determination for the compound E was done through instrumental analysis including UV, IR, NMR to compound E, its genin and derivatives. The results obtained from spectral analylsis showed that the moiety of compound E was luteolin glycoside attached with glucose at the position of 7th carbon of compound. Therefore, the compound E which is separated and purified from *Lactuca dentata Makino* was identified as luteolin-7-0-β-D-glucoside.

V. 요 약

한국산 씀바귀(*Lactuca dentata Makino*)의 ethyl acetate 추출물에서 6 종류의 flavonoid 성분이 TLC에 의해서 검출 되었으며, 이들은 극성에 따라서 A에서 F가지 명명되었다. 그들 중 주성분으로 생각되는 물질 E를 column chromatography을 이용하여 분리정제 하였다. 물질 E의 녹는 점은 249.5℃-251℃로 나타났으며, 그 구조를 밝히기 위하여, 물질 E와 이를 가수분해한 genin에 대한 여러가지 shift reagent에서 UV와 IR 분석을 하고 또한 물질 E와 그의 acetyl 유도체에 대한 NMR 분석을 하였으며 물질 E에 결합된 당은 TLC에 의하여 확인하였다. 이와같은 분석 결과에서 물질 E는 luteolin 모핵에 glucose가 결합된 luteolin-7-0-β-D-glucoside로서 확인되었다.

References

1. Chung, T.H. 1972. Encyclopedia of Korean plants, p. 746, Seoul, Korea.
2. Lee, D.B. 1974. Encyclopedia of Korean plants and animals. p. 298, Seoul, Korea.
3. Mark, E.M. and Stewar, G.F. 1954. Advanced in food research **5**: 262.
4. Kuhnau, J. 1973. Die flavonoide und ihre rolle in der Menschlichen Ernahrungm ein Beitrag zur Kenntnis Semi Essentiellar Pflanzenstoffe, Qual-Pl. Fds. Hum. Nut. **23**: 119.
5. Mabry, Tom J. and Uivelen. 1980. Chemistry and utilization of phenylpronoids including flavonoid, coumarins and lignans. J. Agric. Food Chem. **28**: 188.
6. Motin Woldecke und Karl Herrman. 1974. Flavonole und flavonone der Gemusearten, Z. Lebensm. Unter Forsch **156**: 153.
7. Makoto, Kaneta., Hirashi Hikichi, Seiichi Endo and Noboru Sugiyama. 1978. Identification of flavonones in sixteen *Compositae species.* Agric. Biol. Chem. **42**: 478.
8. Chao tung Chen and Kun-Lan Chang. 1976. A study on the constitutes of *Lactuca chinensis Makino.* Bull. Inst. Chem. Acad. Sin. **23**: 31.
9. Tozaburo, Kurihara and Kikuchi, Masao. 1975. Studies on the constituent of flowers, IV. on the component of flower of Viburnum dilatatum THUNB. Yakugaku Zasshi **95**: 1098.
10. Tashichio, Nakaoki and Naokata, Morita. 1960. Studies on the medicinal resources XVI. Yakugaku Zasshi **80**: 1473.
11. Park, S.S. 1977. Biological activity of *Ixeries sonchifolia H.* Korean Biochem. J. **10**: 241.
12. Yun, S.K. 1973. Chemical study on cookery of an edible wild glass *Ixeris dentata.* Andong Educational University. Thesis(II) **7**: 279.
13. Marby, T.J., Markham, K.R. and Thomas, M.B. 1970. The systematic identification of flavonoid. pp. 35-60 and 254-273. Springer-Verlag, New York, USA.
14. Challice J.S. and William A.H. 1968. Phenolic com-

pounds of the Genus pyrus-II. Phytochemistry **7**: 1781.
15. Ulubelen, A. and Doguc, T. 1974. Flavonoid compounds from the flower of *Genista Lydia*. Planta Medica **25**: 39.
16. Jacques Kagan. 1968. Luteolin-7-glucoside, the flavonoid pigment of *Heliotropium tenellum*. Phytochemistry **7**: 505.
17. Tsuyoshi, Tommimori, Yoshizaki, Masao and Nanba, Tsuneo. 1973. Studies on the Nepalese crude drugs. I. on the flavonoid and xanthone constituents of the plants of *Swertia spp*. Yakugaku Zasshi **93**: 442.
18. Sinha, N.K., Seth, K.K., Pandey, V.B., Dasgupta, B. and Shah, A.H. 1981. Flavonoid from the flower of *Clerodendron infortunatum*. Planta Medica **42**: 296.