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PHYTOCHEMICAL ADAPTATION OF COASTAL VEGETATION IN TAIWAN

I. Isolation, Identification, and Biological Activities of Compounds in *Vitex negundo* L.[1,2]

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Abstract

Vitex negundo is a dominant coastal vegetation and widely distributed in the southern parts of Taiwan. The biomass and density of its associated understories are relatively lower than its adjacent pasture. The field experimental results showed that the natural leachate of *V. negundo* significantly retarded the growth of *Digitaria decumbens* but stimulated the growth of *Andropogon nodosus* as compared to the rainfall control. The growth of *D. decumbens* grown in pot under greenhouse conditions was significantly retarded by watering a 1% aqueous extract of *V. negundo*, but the growth of *Andropogon nodosus* and *Mimosa pudica* was stimulated. The aqueous extract also revealed remarkable phytotoxicity when lettuce and rye grass seeds were used as test material, comparing the distilled water control. The aqueous effluents obtained from a polyamide column chromatograph were also bioassayed, and the results showed that fractions 1, 2, 3, and 10 exhibited inhibition of radicle growth of lettuce and rice seedlings, while fractions 7 and 9 revealed stimulation effect. The responsible substances were isolated and identified. These include phenolic acids, *p*-hydroxybenzoic, ferulic, *p*-coumaric, vanillic, and syringic acids, and 10 flavonoids. Due to insufficient quantity of isolated flavonoids, we were unable to do some detailed spectrometric analyses, such as NMR and GC-mass. Most of isolated flavonoids were preliminarily assigned by their UV-visible spectra. Only one flavonoid, 3′-hydroxy-vitexin, was structural confirmed. Further structural elucidation of other flavonoids present in *V. negundo* is in progress.

Introduction

The adaptation of plants to environmental stresses is often ascribed to the

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morphological and anatomical changes of plants. Leaves of many xerophytes have special structural adaptations that decrease transpiration. For example, leaf of *Nerium* has thick cuticles and stomata with trichomes; leaf of *Agave* also has thick cuticle and outer cellulose wall of epidermis; and leaf of *Poa* shows method of folding when a water deficit develops. These plants with thick cutin or wax on leaf surface reveal a surprising resistance to desiccation under extremely drought conditions (Daubenmire, 1967). Besides structural adaptation, functional adaptation including physiological or phytochemical responses to environmental stresses has received enough attention. Harborne (1982) made an extensive review on the aspect of biochemical adaptation of plants to environmental stresses and indicated that most of tropical and subtropical grasses, such as Eragrastoideae, Panicoideae and Arundinoideae, have a Hatch-Slack pathway in performing high photosynthetic activity in hot climate. *Vitis vinifera* produces secondary metabolites, such as abscisic acid, phaseic acid, *trans*-farnesol, and xanthoxin, which play an important role in stimulating stomata closure (Loveys and Kriedemann, 1974; Wellburn *et al.*, 1974). Some halophytes are able to accumulate proline and betaine in regulating osmotic pressure of guard cell to reduce transpiration (Stewart and Lee, 1974). On the other hand, phenolics and flavonoids may be produced in a relatively high concentration by plants which exposed to high intensity of UV radiation or to saline environment.

In the subtropical and tropical areas of Taiwan, there is luxuriant costal vegetation under drought, saline, and high UV radiation conditions. Little information concerning the phytochemical adaptation of the vegetation is known, thus a series of study has been undertaken in this aspect. We here report the first step study on isolation, identification, and biological activities of phenolic acids and flavonoids isolated from *Virtex negundo*, a common species distributed in the coastal area of southern Taiwan.

Materials and Methods

Materials

Leaves of *Vitex negundo*, collected from the hillsides of Hengchun County, Taiwan, were brought to the laboratory of Institute of Botany, Academia Sinica. The leaves were air-dired at room temperature, and chopped into small piece before analysis.

Field Experiments

The field experiment was set at the experimental farm of Hengchun Livestock Experiment Station. Quadrates, 1×1 m for each, were set in the pasture of *Digitaria decumbens* and *Andropogon nodosus*. Each quadrat was iron-fenced to

avoid animal disturbance. The top of fence, 1.5 m above the ground, was placed with 3 kg of fresh plant parts with leaves. The grasses in the test and control quadrates received equal physical treatment, thus no physical competition existed between the test and control experiments. The experiment was arranged according to a randomized block design with four replicates. The plot receiving raindripping through *V. negundo* plant covers was designated as raindrip plot, and the plot receiving natural raindrip served as the control. The grasses received either raindrip through *V. negundo* (called leachate) or rainfall were harvested at the end of one month, and the dry weight was measured. The experiment continued for five months.

Greenhouse Pot Experiment

The stolon cuttings of *D. decumbens, A. nodosus,* and *Mimosa pudica* were planted in pots (5,000/are) with vermiculite as substrate. These plants were grown healthily in the greenhouse of Academia Sinica for one month. The upper 10 cm portion of these plants was cut out and then separately irrigated with distilled water or 1% aqueous extract of *V. negundo.* The experiment was set up in four replicates. On the 30, 60, and 90th day after irrigation, plants were harvested and their dry weights measured.

Preparation of Aqueous and Methanolic Extracts of Leaves

To 100 gram dry ground leaves of *V. negundo,* 1,900 ml of distilled water was added and the mixture was shaken for 2 h. The aqueous extract was filtered through Whatman 42 filter paper, and the residue was added with the same amount of distilled water and shaken for 2 h. The subsequent extracts were obtained by suction filtration. The aliquots from each extract were taken for bioassay, and the remaining portions were combined for concentration *in vacuo* at temperature below 50°C. The final syrup like solution was used for isolating flavonoids by column chromatography, and the condensate was stored in a cold room at 5°C for other assays. To the residue of aqueous extraction was added a subtantial amount of methanol, and the methanol extraction was repeated several times till the extract became colorless. All methanolic extracts were combined and further concentrated to a syrup like solution for column chromatography.

Bioassay of Aqueous Extract of Leaf

The aqueous leaf extract of *Vitex negundo* was bioassayed by techniques described previously (Chou and Young, 1975; Chou and Lin, 1976). Seeds of *Lactuca sativa* var. Great Lakes and *Lolium multiflorum* were used as test material. The germination percentage and radicle growth in millimeter were measured after 72 h incubation at 25°C.

Isolation of Flavonoids and Phenolic Compounds

About 150 g polyvinylpolypyrrolidone powder (purchased from Sigma Chemical Company, USA) was soaked with 1,500 ml double distilled water or reagent grade methanol. The mixture was stirred thoroughly and allowed to settle overnight. The turbid supernatant was decanted and re-added with the solvent. This process was repeated three times till the supernatant was clear. The slush of polyvinylpolypyrrolidone was packed into a glass column, 8×90 cm, and allowed to settle gradually. The 60-mesh clean sea sand was evenly covered on the top of polyvinylpolypyrrolidone to about 1 cm in depth. Aliquot of the syrup-like extract was discharged into the column, and was eluted with a step-up solvent from 100% distilled water to 100% methanol. Eluate of each fraction was concentrated and chromatographed on a polyamide thin-layer sheet (purchased from Cheng Chin Trading Co., L. T. D., Taiwan). The TLC sheet was developed with solvents: (1) methanol-acetic acid (9:1, v/v) (Harborne, 1973), (2) benzene: MEK: MeOH (40:30:30, v/v/v), (3) $CHCl_3$: MeOH: MEK (9:4:1, v/v/v), and (4) H_2O: MEK: MeOH: acetylacetone (13:3:3:1, v/v/v/v) (Newman *et al.*, 1979). Two dimensional paper chromatography was also employed to purify the eluate of flavonoids by solvents, namely TBA and 15% acetic acid, described by Mabry *et al.* (1970). After several times of re-chromatography, the isolated compound became relatively pure. The compound was finally cleaned by passing it through a Sephadex LH 20 column and eluted with spectroscopic grade methanol. The eluate was concentrated to a small volume and placed in a refrigerator to allow crystallization. On the other hand, phenolic acids were isolated by method described by Chou and Young (1975).

Identification of Phenolic Acids and Flavonoids

The isolated phenolic acids were identified by paper and thin-layer chromatography using authentic compounds as reference. The isolates of flavonoids were analyzed by a UV-visible spectrophotometer (Hitachi model 100-50), nuclear magnetic resounance spectrometers (Jeol FT-100), and gas chromatograph-mass spectrophotometer (Jeol JMS D-100).

Results

Effects of Leachate and Aqueous Extracts of ***Vitex negundo*** *on Plant Growth*

In the field, the growth performance of two pastures, namely *Andropogon nodosus* and *Digitaria decumbens*, affected by natural rainfall and the raindrip (the leachate of *V. negundo*) was compared. The results are shown in Fig. 1, indicating that the growth and biomass of *D. decumbens* was significantly suppressed but that of *A. nodosus* was stimulated by the leachate of *V. negundo* compared to those of

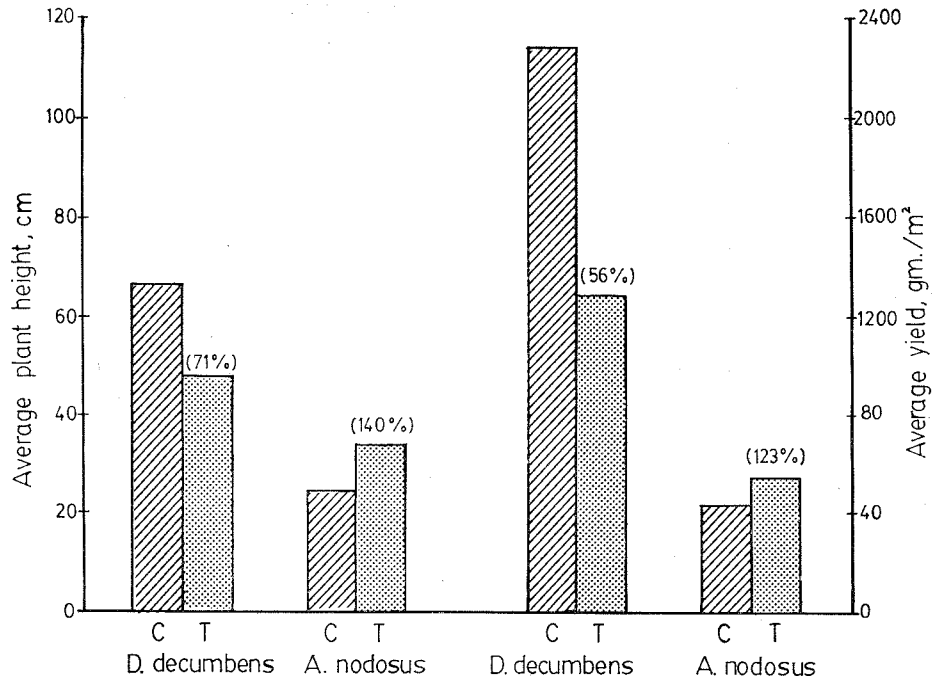

Fig. 1. Effects of natural leachate of ***Vitex negundo*** (T) on the growth of pastures, ***Digitaria decumbens*** and ***Andropogon nodosus*** in field, compared to that of rainfall control (C). Data in parentheses indicate the index of T/C×100%.

rainfall control.

Cuttings of *Mimosa pudica* as well as the above two pastures were grown in pot under greenhouse conditions. These plants were irrigated with 1% aqueous extracts or distilled water. The results showed that the growth of these plants was obviously stimulated by the extract harvested after 60 days of treatment compared to that of the distilled water control. Only on the early 30-day growth period, the growth of *D. decumbens* was retarded (Fig. 2). It is necessary to note that the relative growth stimulation revealed by the extract treatment may not be fully due to the growth promoting substance but due to the nutrient effect of the extract. As control, the distilled water revealed neither stimulation nor inhibition on the weed growth.

Regarding the laboratory bioassay, the 5% aqueous extract and its subsequent extracts were bioassayed by using lettuce and rye grass as test materials. The phytotoxicity was estimated by comparing the percent inhibition of growth by extract against distilled water control. The extract showed a significant inhibition of seed germination and radicle growth regardless of test species (Fig. 3). The inhibition was evidently found in the first extraction and decreased in the subsequent extractions. The inhibition of lettuce seed germination was significantly greater

than that of rye grass, while the inhibition of radicle growth was the reverse. Furthermore, the extract was diluted to 1%, 2%, 3% and with distilled water and bioassayed by lettuce seeds. The bioassay results are shown in Fig. 4, indicating that the inhibition decreased with dilution. The osmotic concentration of 4% extract was too low (below 25 milliosmols) to cause an osmotic effect, thus the inhibition was due to phytotoxins present in the extrcts.

Bioassay of Aqueous Eluate Fraction from Column Chromatograph

The concentrated aqueous extract was discharged into the column as mentioned in the previous section of this paper, and then was eluted with distilled water. We were able to locate bands on column under a UV viewer in the dark, thus each band was separately collected and designated as fractions 1 to 12. Eluate of each

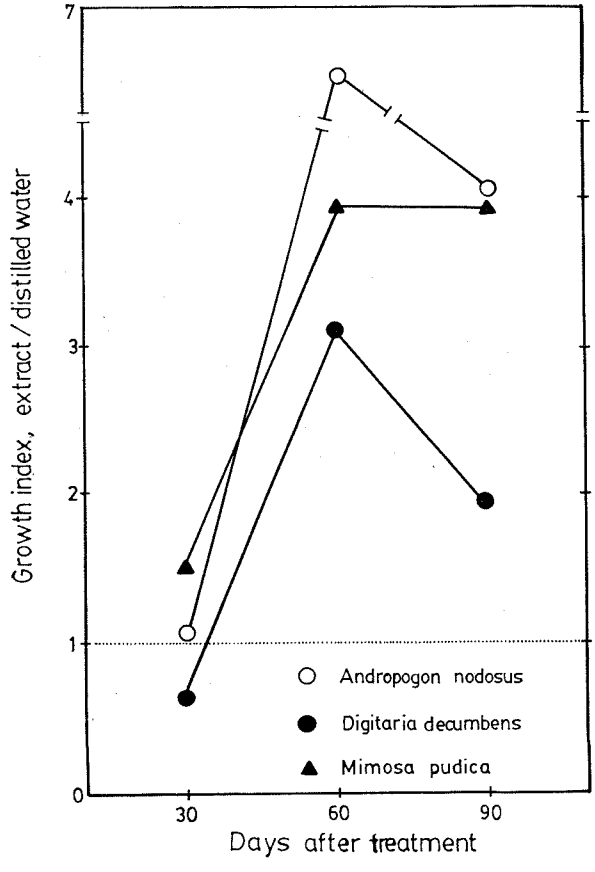

Fig. 2. Effects of aqueous extract of *V. negundo* on the growth of *Andropogon nodosus*, *Digitaria decumbens*, and *Mimoa pudica* grown in pot under greenhouse conditions.

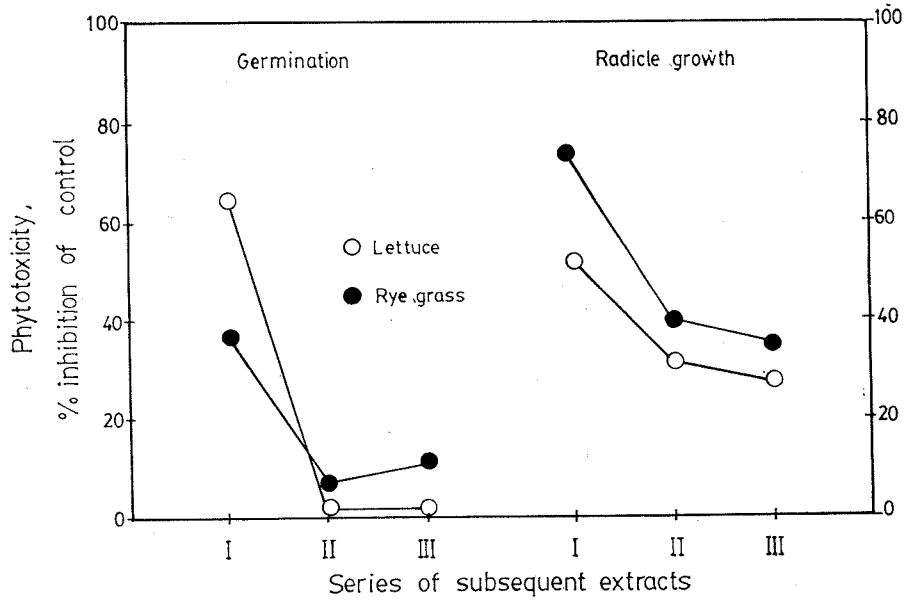

Fig. 3. Phytotoxic effects of 5% aqueous extracts in three subsequent extractions from leaves of *V. negundo* on the seed germination and radicle growth of lettuce and rye grass.

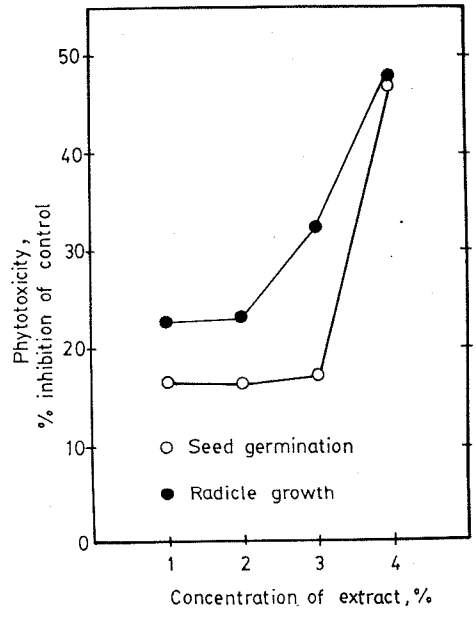

Fig. 4. Relative inhibition of aqueous extracts in 4 concentrations on the seed germination and radicle growth of lettuce seedlings.

fraction was bioassayed by lettuce. The bioassay results are shown in Fig. 5. It was found that fractions 1, 2, 3, and 10 revealed significant inhibition on radicle growth while fractions 7 and 9 exhibited stimulation. The phytotoxic compounds as well as stimulating substances present in the aqueous fractions were also collected for structural identification reported in the following sections.

Identification of Phytotoxic Substances from Leaves of ***V. negundo***

The aqueous eluate of column chromatograph was concentrated to a small volume and re-extracted with ether in order to identify the responsible growth substances. By means of paper and thin-layer chromatography, five phytotoxic phenolics were identified. These include ferulic, *p*-hydroxybenzoic, *p*-coumaric, vanillic, and syringic acids (Table 1). These five compounds are mostly present in the fraction 3 of column chromatograph (Fig. 5), and relatively small amount was present in fractions 1 and 2. However, the stimulating compounds eluted from column chromatograph were flavonoids in nature. The flavonoids were

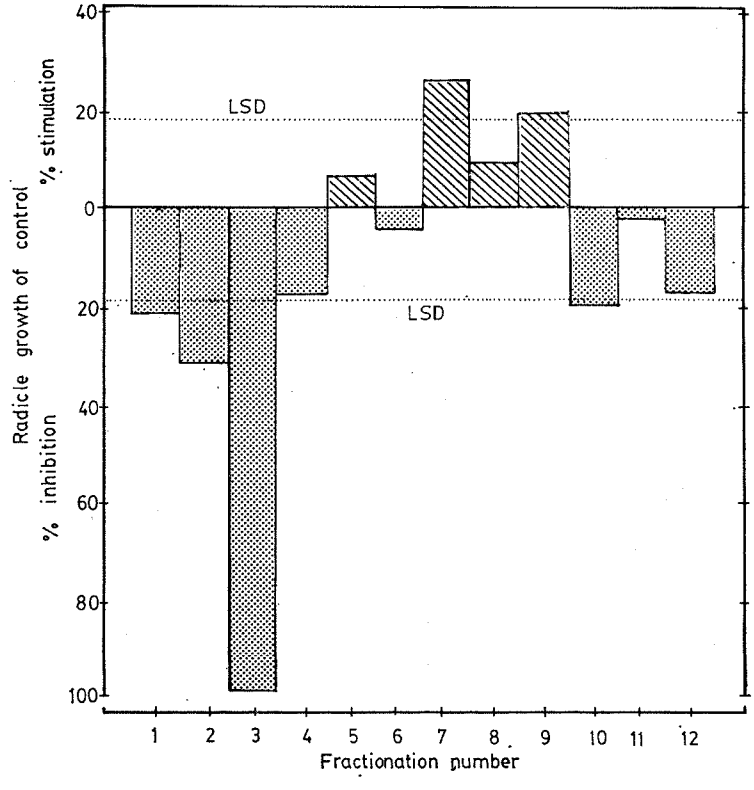

Fig. 5. Effects of eluates from the aqueous fraction of column chromatograph discharged by aqueous leaf extracts of *V. negundo* on the radicle growth of lettuce seedlings.

Table 1. *Phytotoxic phenolics isoloted from aqueous leaf extract of* ***Vitex negundo***

Compound	Rf (2% HOAc)	Color reaction	
		sUV(1)	DPNA(2)
Ferulic acid	0.39	blue fl	sky blue
p-Hydroxybenzoic acid	0.68	purple ab	red
p-Coumaric acid	0.47	purple ab	deep blue
Vanillic acid	0.63	purple ab	violet
Syringic acid	0.56	blue fl	blue

(1) sUV: 254 nm of UV light
(2) DPNA: spray with diazotized *p*-nitroaniline followed by 20% Na_2CO_3
(3) ab = absorption, fl = fluorescence

isolated not only from the aqueous fractions as shown in Fig. 5 but also from the methanolic fractions after the aqueous elution of column chromatograph.

Preliminary Identification of Flavonoids in ***V. negundo***

Ten relatively pure isolates of flavonoids were obtained from the aqueous and methanolic extracts of leaves of *V. negundo* by means of column chromatography. According to the systematic identification systems described by Mabry *et al.* (1970) and Markham (1982), one of them was structurally identified (Compound I of Fig. 7) and the remaining compounds were tentatively identified (Compound II to Compound X in Fig. 7). The paper chromatographic data of each isolate are given in Table 2, and the data of UV-visible spectrophotometric analysis are shown in Table 3. In addition, the NMR spectrum of Compound I is shown in Fig. 6. Combining the data of PC, TLC, UV-visible spectrometric, and NMR analyses, Compound I was identified as 3′-hydroxy-vitexin. However, due to insufficient quantity of the remaining nine isolates, we were unable to do the NMR study and the sugar analysis of these compounds. from the data of paper chromatography of each compound (Compound II, III, V, VII, IX, and X) as shown in Table 2, the R_f values of the compounds ranging from 0.31 to 0.58 of the first developing solvent (TBA) and from 0.10 to 0.58 of the second solvent (15% HOAc) indicate that these compounds have a pyranosyl sugar linked to either C-7-O or C-8 positions according to the findings of Mabry *et al.* (1970). According to the spectra of UV-visible spectrometric analysis of the compounds, there are OH and OCH_3 attached to C-5, C-7, C-3, C-3′, C-4′ and OR attached to C-3 position. The detailed study of these compounds needs to be done.

Preliminary study of the biological activity of these compounds was also conducted, yet inconsistent results of inhibition and stimulation on the radicle growth

Table 2. *Paper chromatography of flavonoids isolated from* ***V. negundo***

Compound	Rf(1)		Color reaction(2)		
	1st	2nd	UV	UV/NH_3	UV/NA
I	0.57	0.31	purple	yellow	orange
II	0.49	0.58	purple	yellow	yellow
III	0.35	0.52	purple	yellow	yellow
IV	0.58	0.19	purple	yellow	orange
V	0.67	0.51	purple	yellow	yellow
VI	0.13	0.42	purple	yellow	yellow
VII	0.31	0.13	purple	yellow	yellow
VIII	0.76	0.05	purple	yellow	yellow
IX	0.33	0.17	purple	yellow	yellow
X	0.54	0.10	purple	yellow	yellow

(1) 1st dimension TBA=*t*-butyl alcohol: acetic acid: water (3:1:1, v/v/v)
2nd dimension 15% HOAc=acetic acid: water (15:85, v/v)
(2) UV: long wavelength UV light (366 nm)
UV/NH_3: chromatogram fumed with ammonium vapor and viewed under UV light
UV/NA: chromatogram sprayed with NA reagent and viewed under UV light

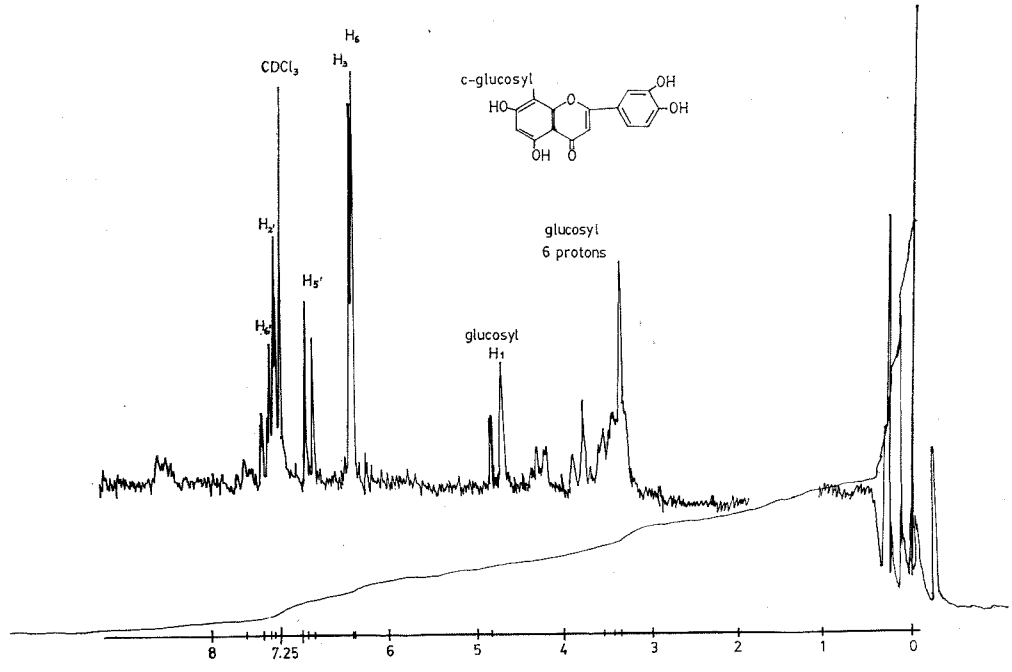

Fig. 6. The NMR spectrum of 3′-hydroxy-vitexin isolated from leaf of *V. negundo*.

Table 3. *UV-visible absorption spectra of flvonoids isolated from* ***V. negundo***

Data was shown by absorption in nm.

Compound	MeOH	MeOH + NaOMe	MeOH + $AlCl_3$	MeOH + $AlCl_3$ + HCl	MeOH + NaOAc	MeOH + NaOAc + H_3BO_3	MeOH + HCl
I	255sh 267 346	267 335sh 404↑	273 301sh 325sh 421	261sh 276 292sh 353	265 325 403	265 374 432sh	255 270 347
II	269 300	269 300sh 388↑	274 295sh 346 380sh	275 297sh 340 377sh	269 331sh 387	268 331	267 325
III	271 331	281 331sh 395↑	276 305sh 343 383sh	278 298 339 384sh	280 332sh 393	270 306sh 325 353sh 403sh	270 329
IV	255 267 344	267 330 406↑	273 299sh 420	260 275 350 390sh	268 325sh 401	263 372 430sh	255 345
V	272 330	276 325 390↑	276 302sh 346 383sh	275 302sh 341 380sh	272 330sh 383	272 313sh 325 335sh	272 330
VI	260 332	273 331sh 398↑	272 303sh 345 390	259 267sh 300sh 337 390sh	266 386	263 350 436sh	260
VII	253 268sh 340	266 328sh 399↑	269 301sh 325sh 418	257 294sh 347 390	261 389	258 364 429sh	253 340
VIII	255 274sh 281sh 343	264 324sh 396↑	272 295sh 325sh 421	260 272sh 293sh 351 380	267 325sh 390	260 370 432sh	255 274sh 281sh 343
IX	253 276sh 346	264 395↓	272 297sh 325sh 420	259 296sh 350 385sh	259 400	257 367	253 267
X	269 334	253sh 273 326sh 388↓	250 277 295sh 343 388sh	248sh 270 295sh 343 388sh	273 307sh 324sh 388	269 337	237sh 269 340

(1) abbreviation: ↑ = intensity increases
↓ = intensity decreases
sh = shoulder

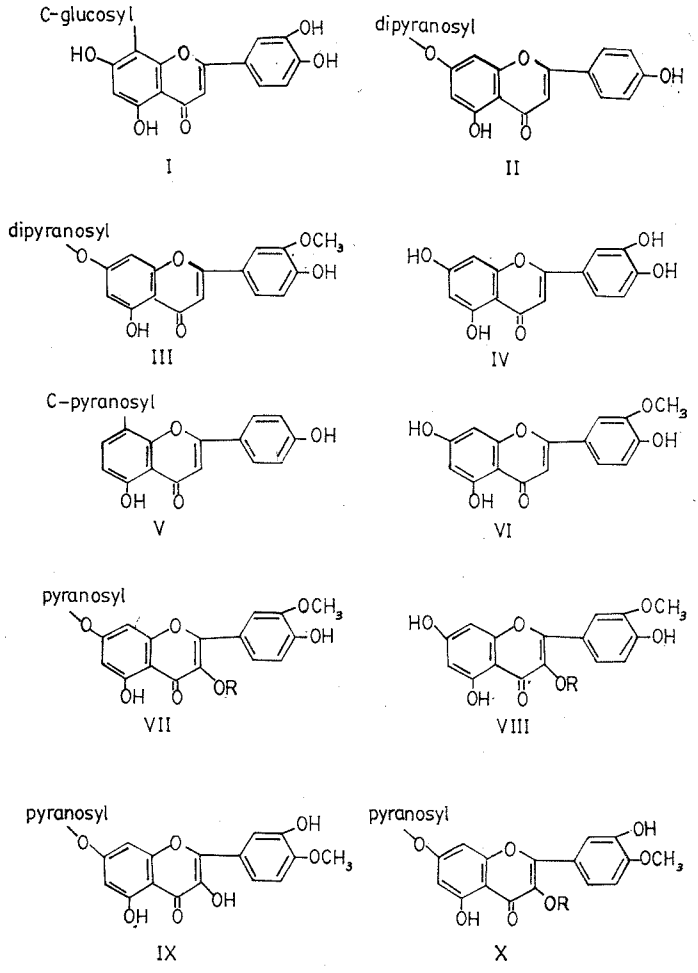

Fig. 7. Tentative structures of flavonoids isolated from *V. negundo*.

of lettuce were found, indicating that the possibility of breakdown of flavonoid glycoside into aglycone occured during the course of various assays (Harborne *et al.*, 1975). Further studies are needed to clarify this problem.

Discussion

Plant secondary metabolites produced through *de novo* synthesis are of great importance in the adaptation of species and in the organization of plant population (Whittaker and Feeny, 1971; Chou and Waller, 1983). The quantity of the metabolites, such as phenolic acids, flavonoids, alkaloids, and terpenoids is usually higher when plants are under stress conditions than the normal conditions (Muller, 1969; Koeppe *et al.*, 1966, ; Waller and Nowacki, 1978). Harborne (1982) indicated

that some coastal vegetation often contains relatively higher amount of flavonoids and other groups of compounds, such as betaine or proline. *Vitex negundo* has adapted to the coastal environment of Taiwan for many centuries, and has formed a dominant vegetation. This plant produces some volatile compounds, which manifest deleterious effect upon mosquitoes (Hung *et al.*, 1976) and some fruit flies (1982, Chou unpublished data). On the other hand, *V. negundo* also releases water soluble compounds, five phenolic acids and 10 flavonoids, which exhibited both inhibitory and stimulatory effects on some herbaceous plants tested (Figs. 1 to 5). Among these flavonoids, 3′-hydroxy-vitexin was present in a high amount (about 6 mg/200 g leaves), while the remaining flavonoids were present in very low quantity. We were unable to determine the sugar moiety and phytotoxic activity of each isolated flavonoids. Additionally, the fruit of *V. negundo* has phamaceutical potential as anti-cold, headache, neuralgia, and stomachache (Hung *et al.*, 1976). We have attempted to continue the isolation and identification of the natural products from *V. negundo*, and hopefully, may find further significant results concerning the biological activity of flavonoids isolated.

In summary, the present findings of isolation and identification of phenolic acids and flavonoids revealed at least that *V. negundo* produces subtantial quatity of natural products, which might play an important role in the adaptation of coastal vegetation, although the mechanism still needs to be further studied.

Acknowledgement

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Literature Citeed

Chou, C. H. and H. J. Lin. 1976. Autointoxication mechanism of *Oryza sativa* I. Phytotoxic effects of decomposing rice residues in soil. J. Chem. Ecol. 2: 353-367.

Chou, C. H. and C. C. Young. 1975. Phytotoxic substances in twelve subtropical grasses. J. Chem. Ecol. 1: 183-193.

Chou, C. H. and G. R. Waller (eds.). 1983. Allelochemicals and Pheromones. Institute of Botany, Academia Sinica Monograph Series No. 5. Taipei, R. O. C., 324 pp.

Daubenmire, R. F. 1967. Plants and Enviornment. John Wiley & Sons, Inc., New York, London, Sydney, 422 pp.

Harborne, J. B. 1973. Phytochemical Methods. Chapman and Hall, London, pp. 33-88.

Harborne, J. B. 1982. Introduction to Ecological Biochemistry, 2nd edn. Academic Press, London, New York, 278 pp.

Harborne, J. B., T. J. Mabry, and H. Mabry (eds.). 1975. The Flavonoids. Chapman and Hall, London, 1204 pp.

Hung, T. S., C. L. Mon, Y. C. Lee, and M. I. Chen (eds.). 1976. Seaside Plants of Taiwan. Published by the CADP, R. O. C. (in Chinese), 333 pp.

Koeppe, D. E., L. M. Southwick, and J. E. Bittell. 1976. The relationship of tissue chlorogenic acid and concentrations and leaching of phenolics from sunfiowers grown under varying phosphate nutrient conditions. Can. J. Bot. **54**: 593-599.

Loveys, B. R. and P. E. Kriedemann. 1974. Internal control of stomatal physiology and photosynthesis I. Stomatal regulation and associated changes in endogenous levels of abscisic and phaseic acid. Aust. J. Plant Physiol. **1**: 407-415.

Mabry, T. J., K. R. Markham, and M. B. Thomas. 1970. The Systematic Identification of Flavonoids. Springer Verlags, Berlin, 253 pp.

Markham, K. R. 1982. Techniques of Flavonoid Identification. Academic Press, London, New York, Paris, San Diego, San Francisco, 113 pp.

Muller, C. H. 1969. Allelopathy as a factor in ecological process. Vegetatio **18**: 348-357.

Neuman, P. R., B. N. Timmerman, and T. J. Mabry. 1978. Laboratory Manual for the Systematic Identification of Flavonoids. MS, University of Texas at Austin.

Stewart, G. R. and J. A. Lee. 1974. The role of proline accumulation in halophytes. Planta **120**: 279-289.

Waller, G. R. and E. K. Nowacki. 1978. Alkaloid Biology and Metabolism in Plants. Plenum Press, New York, London, 294 pp.

Wellburn, A. R., A. B. Ogunkanmi and T. A. Mansfield. 1974. All *trans*-farnesol: a natural occurring antitranspirant? Planta **120**: 255-263.

Whittaker, R. H. and P. P. Feeny. 1971. Allelochemics: Chemical interactions between species. Science **171** (3973): 757-770.

臺灣沿岸植被之植物化學適應

一、蒲姜中生物活性物質之分離、純化及鑑定

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蒲姜 (*Vitex negundo*) 是本省恒春一帶之主要沿岸林，在此植被下，其地被植物之生物量及密度較毗鄰的草原區者爲低。田間實驗結果顯示，蒲姜葉的雨淋洗液對當地之盤固草 (*Digitaria decumbens*) 的生長有顯著的抑制作用，但對藍莖草 (*Andropogon nodosus*) 具促進作用。將盤固草，藍莖草及含羞草移植於盆栽中並置於本所之溫室中，並分別澆以1%蒲姜葉之水溶萃取液及自來水（對照組）比較得知，該萃取液對前者之生長有抑制作用，但對後兩者却有促進作用。取5%蒲姜葉之水溶萃取液對萵苣及裸麥草之種子發芽及幼根生長具抑制作用。此水溶萃取液經管柱色層分離管並以水淋洗之得十個不同的濾出液，此濾出液對萵苣之生長亦有促進及抑制作用。進一步的分離純化得十五個純化物，其中有五個是酚酸化合物經鑑定得知是 *p*-hydroxybenzoic, *p*-coumaric, ferulic, vanillic 及 syringic acids，另十個爲類黃素，其中一個所得量較多經紫外光—可見光譜儀，核磁共振儀及氣析—質譜儀之分析鑑定爲 3′-hydroxy-vitexin；另外九個類黃素之取量均低故僅能做紫外光—可見光譜儀之分析，其詳細構造式有待進一步之研究以確定。