Natural Product Sciences
2(1) : 37 ~ 42 (1996)

Antiplatelet Aggregation Potencies of some *Allium* spp. Grown in Indonesia

C. Hanny Wijaya[1], Deddy Muchtadi[1], Herianus J. Lalel[2], Fransiska Zakaria[1], and Sutrisno Koswara[1]

[1]Dept. of Food Tech. and Human Nutr., FATEAT, Bogor Agriculture Univeristy (IPB), Bogor 16002 Indonesia
[2]Fac. of Agric. Nusa Cendana University, Kupang 85119, Indonesia

Abstract – Several species and varieties of *Allium* spp. grown in Indonesia were screened for their *in vitro* antiplatelet aggregation activities. The extracts were also analyzed by GC for their volatile sulfur-containing compounds, and measured for their Volatile Reducing Substances (VRS) contents. Antiplatelet aggregation potencies of *Allium* spp. were varied among the species and varieties studied. Garlic extracts showed the greatest ability to inhibit platelet aggregation followed by extract of shallot, chive, yellow onion, and green onion. The "Jawi" and "local Padang" variety of garlic showed high activities with IC_{50} values of 9.1 and 9.8 μg/ml, respectively. The local Kupang variety of shallot showed the highest antiplatelet activity among the shallot varieties evaluated, with an IC_{50} value of 111 μg/ml. Antiplatelet aggregation activities of *Allium* extracts showed a positive correlation with the level of volatile compounds.

Key words – antiplatelet-aggregation, *Allium* spp, Indonesia.

Introduction

The family health survey carried out by The Indonesia Ministry of Health in 1986 showed that the incidence of coronary heart disease in Indonesia had reached 508 per 100,000, with an attendant mortality rate of 65 per 100,000. By 1992, coronary heart disease had become the primary cause of mortality in Java and Bali.

Consumers are becoming increasingly concerned with functional health foods (Tokoro, 1978). Potential health benefits of *Allium* include hypocholesterolemic (Bordia *et al.*, 1975) and antithrombotic (Bordia, 1978 ; Apitz-Castro *et al.*,1983 ang Block *et al.*, 1986) activities. Garlic and onion products posses flavor compounds such as methyl allyl trisulfide (Ariga *et al.*, 1981), alliin, allicin (Kyriakides, 1985), vinyldithiins (Nishimura *et al.*, 1988), ajoene and diallyl trisulfide (Apitz Castro *et al.*, 1983 and Block *et al.*, 1986). These compounds have been shown to be capable of preventing vena clogging and reducing the proliferation of lipid plagues.

Many species of *Allium* are widely cultivated in Indonesia, most of which are used as seasoning. These include garlic (*A. sativum L.*) which has several varieties (e.g. *A. sativum* var. *sativum* and *A. sativum* var. *pekinense*), shallot (*A. cepa* var. *aggeratum*), chive (*A. schoenoprasum*), yellow onion (*A. cepa* var. *cepa*), and green onion (*A. ampeloprasum* var. *B. Porum*) (Ochse and van den Brick, 1931).

This research was conducted to obtain a preliminary evaluation of the *in vitro* antiplatelet aggregation activities of a number of *Allium* species that are generally cultivated in Indonesia.

Experimental

Raw material – Shallot samples were obtained from various locations in Indonesia including Brebes (varieties of Bangkok, Kuning Gombong and Filipina), Malang (variety of Batu), and Nusa Tenggara (variety of Local Kupang); garlic samples were obtained from Central Java (varieties of Jawi and Lengkong), West Java (variety of Lombok Ijo), West Sumatra (variety of Local Padang), and Nusa Tenggara (variety of Local Belu); chive, yellow onion and green onion were obtained from Cipanas.

Antiplatelet aggregation analysis – Antiplatelet aggregation activities were analyzed by an *in vitro* method (Apitz-Castro *et al.*, 1983 and Kyriakides, 1985).

Sample preparation – An extract of each *Allium* tissue was prepared by homogenization with methanol (1:1,w/v). After incubation at 30-36 °C for 30 min, methanol was evaporated from the homogenate at 50 °C and the resulting aqueous phase was extracted with diethyl ether. The ether phase was washed three times with water, dried over anhydrous sodium sulfate and evaporated under vacuum. The oily residue was dissolved in methanol and kept at -20 °C (Apitz Castro *et al.*, 1983).

Plasma preparation – White New Zealand (F2) Rabbit's blood was collected from ear vena with a sterile plastic syringe. Nine parts of blood were mixed with one part 3.8% sodium citrate and centrifuged at 160 g for 10 min to yield platelet rich plasma (PRP) in the upper phase (supernatant). The remaining blood specimen was further centrifuged at 200 g to give platelet poor plasma (PPP).

Platelet aggregation analysis – Platelet aggregation induced by ADP was measured at 37 °C in an Aggrecoder (model PA-3210, Daichi co.) using a turbidometric.

Allium extracts were diluted with PPP to obtain the desired concentration, and then 10 μl of this diluted sample was added to 440 μl PRP. After incubation for 90 sec, 50 μl of 10 μM ADP was then added. Antiplatelet aggregation activity was expressed as IC_{50} value that was capable of inhibiting platelet aggregation by 50% relative to a control sample void of *Allium* extract.

Volatile compounds analysis – The sum of volatile compounds in *Allium* extracts were analyzed by the volatile reducing substances (VRS) method based on aeration and titration (Farbor, 1967). The volatile substances especially sulfur-containing compounds were identified by GC and GC-MS. Comparison with GC profiles derived from similar studies was also done to confirm the identification (Wu *et al.*, 1982; Wijaya *et al.*, 1991; Yu and Wu, 1989).

GC and GC-MS condition – Identification of volatile compounds in methanol extract of *Allium* was carried out using GC (Shimadzu model GC-AM with Carbowax-20M SCOT capillary column (50 m × 0.02 cm and FID detector) and GC-MS (Shimadzu model QP-500, with DB-1 capllary column). The GC condition was as follows: temperature of detector and injector were 225 °C, initial temperature of the column was 40 °C maintained for 1 min and raised 5 °C/min to 180 °C, then held for 5 min. The column condition for GC-MS unit was initially 100 °C and raised 4 °C/min to 200 °C.

Results and Discussion

Antiplatelet activities of *Allium* extracts – The optimum ADP concentration to be used in the assays of antiplatelet aggregation activities was estabilished elsewhere (Wijaya *et al.*, 1994), and it was demonstrated that the optimum concentration of ADP solution was 10 μM (the final ADP concentration in measured platelet 1 μM).

All of the *Allium* extracts showed a similar inhibition of platelet aggregation in a concentration dependent manner. As shown in Tabel 1, garlic extracts had the greatest activity, followed by shallot, chive, green onion, and yellow onion. The average IC_{50} of the five varieties of garlic and shallot were 16 and 420 μg/ml, respectively. According to Morimitsu *et al.* (1992), garlic has greater antiplatelet activity than onion.

Tabel 1 also showed that the antiplatelet activity of *Allium* was not only influenced by their species but also by the variety within each species. Comparing the varieties of garlic, a methanol garlic extract of the Jawi sample showed the greatest anti platelet aggregation activity. It completely inhibited platelet aggregation at a concen- tration of 12 μg/ml PRP. Apitz-Castro *et al.* (1983) reported that methanol extracts of garlic obtained from local markets in Caracas could inhibit platelet aggregation of rabbit blood induced ADP (10 μM) and collagen (2 μg/ml), with a IC_{50} value of 35 μg/ml and 20 μg/ml, respectively.

Antiplatelet aggregation activities of shallot extracts also showed the same phenomena as observed with garlic. There was a rising trend of activities from the shallot variety of Filipina, through variety of Kuning Gombong, Batu, Bangkok to Local Kupang. An extract of the shallot variety Local Kupang had the greatest antiplatelet activity and completely inhibited aggregation at 220 μg/ml with IC_{50} of 111 μg/ml (Tabel 1).

Types, composition, amounts and concentration of sulfur compounds available in fresh *Allium* tissue are likely accountable for the difference of antiplatelet aggregation activities observed in this study. Different species and varieties of *Allium* tissue possess different profile of sulfur components (Whitaker, 1976). Each *Allium* species or varieties has different genetic properties, including its response to changing enviromental conditions, which will further influence the profile of sulfur compounds produced in the tissues. Certain species or varieties have the ability to absorb and use soil nutrients optimally for their metabolism, and then converting them into more complex compounds, including here the precursor compounds responsible for antiplatelet aggregation activities (Fenwick and Hanley, 1985; Freeman and Whenham, 1975). In garlic, the main precursor is allyl-S-cysteine sulfoxide (alliin), while in onion, green onion, chive, and shallot they are 1-

Table 1. IC_{50} value of platelet aggregation inhibition, yield, and VRS value of shallot, garlic, chive, yellow onion, and green onion.

Type of Allium	Variety	IC_{50} (μg/ml)	Extract yield (%, dry basis)	VRS (meq*/g) extract
Shallot	Local Kupang	111	0.05	433.3
	Bangkok	221	0.12	433.0
	Batu	475	0.14	258.2
	Kuning Gombong	606	0.19	242.7
	Filipina	985	0.19	207.3
Garlic	Jawi	9	0.21	10287.0
	Local Padang	10	0.16	8101.0
	Lengkong	17	0.34	5349.0
	Local Belu	19	0.41	10353.0
	Lumbo ijo	24	0.12	3225.0
Chive		799	0.18	525.3
Green onion		870	0.17	351.4
Onion (yellow)		910	0.25	449.9

*microequivalents of potasium permanganate reduced by the volatile material in 1 g of the extract.

propyl-and S-(1-propenyl)-L-cysteine sulfoxide (Freeman and Whenham, 1975; Block, 1985).

It was also reported that allicin, a degradation product of alliin transformation, has antiplatelet activity and is found at different level among the varieties of garlic (Iberl *et al.*, 1990). The allicin concentration of several varieties of crushed garlic from five different countries ranged from 0.12 to 0.545% (wet basis). The spontaneous degradation compounds of allicin, i.e. ajoene, vinyldithiin, methyl allyl disulfide, diallyl trisulfide and 1,5-hexadienyl trisulfide also possess antiplatelet activities (Block, 1985). The synergistic effects (Apitz-Castro *et al.*, 1983) seem to be responsible, in part, for the greater antiplatelet aggregation activity of garlic preparation relative to preparation from other *Allium* spp.

Post harvest handling, climate, and local cultivation practices are other factors that can affect endogenous organosulfur profiles of *Allium* spp. (Fenwick and Hanley, 1985).

Volatile compounds – The content of volatile compounds (VRS) in *Allium* extracts (Tabel 1) appeared to be correlative with their antiplatelet aggregation activities. It is possible that some of these volatile compounds in *Allium* extracts were the putative antiplatelet aggregation agents.

The volatile compounds identified in the methanol extract of the most and least potent antithrombotic of garlic, and the most and least potent antiplatelet aggreagtion of garlic, and the most and least potent antiplatelet aggregation of shallot are presented in Tabel 2 and 3.

The level of six of the seven compounds analyzed in the variety Jawi was greater than in Local Belu (Tabel 2). Among these compounds, five compounds have been reported to be associated with antiplatelet aggregation activity. These include methyl allyl trisulfide (Ariga *et al.*, 1981), 3-vinyl-(4H)-1,2-dithiin, diallyl disulfide, diallyl trisulfide and 2-vinyl-(4H)-1,3-dithiin (Apitz-Castro *et al.*, 1983 and Block *et al.*, 1986). This phenomenon could explain the observation that the garlic variety Jawi had greater antiplatelet aggregation activity than the Local Belu. However, some caution must be exercised in drawing conclusions with GC analysis of organosulfur volatiles. Some of these compounds may be artifacts formed during GC analysis (Block, 1993),

Table 2. Gas Chromatogram area percentages of identified volatile sulfur components on methanol extracts of garlic varieties with the highest and lowest activity.

Components	Area percentage (%)	
	Jawi	Local Batu
Diallyl sulfide	1.24	1.26
Methyl allyl disulfide	4.44	2.50
Diallyl disulfide	6.98	2.03
Methyl allyl trisulfide	1.64	0.34
3-Vinyl-(4H)-1,2-dithiin	19.15	17.49
Diallyl trisufide	42.90	32.91
2-Vinyl-(4H)-1,3-dithiin	15.57	8.47

Table 3. Gas Chromatogram area percentages of identified volatile sulfur components on methanol extracts of shallot varieties with the highest and lowest activity.

Components	Area percentage (%)	
	Lokal Kupang	Kuning Gombong
Propanethion	2.84	2.05
Dimethyl disulfide	1.91	1.66
Propyl 1-propenyl disulfide	1.34	0.85
2,5-Dimethyl thiophene	0.16	nd*
Methylpropyl disulfide	12.06	0.48
3,4-Dimethyl thiophene	0.82	0.39
Methyl cis-1-propenyl disulfide	0.99	0.57
Methyl trans-1-propenyl disulfide	1.29	0.40
Dimethyl disulfide	0.9	nd*
Dipropyl disulfide	3.46	2.13
Cis-1-propenyl propyl disulfide	0.79	0.44
Trans-1-propenyl-propyl-disulfide	1.12	0.73
Methyl propyl trisulfide	3.67	1.67

*nd : not detected

and for that reason it is necessary to establish correlations between organosulfur profiles and antiplatelet aggregation activity by some other method such as HPLC.

There is the possibility that the non volatile organosulfur components from extracts of *Allium* tissue may contribute to the antiplatelet aggregation observed here. Block *et al.* (1986) reported that in garlic, non volatile compounds called ajoene and its analogs have potent antiplatelet aggregation activity. In onion, Morimitsu and Kawashiki (1990) found the similar compound called capaene. Its analogs also have antiplatelet aggregation activity.

In case of shallot, there seems no research correlated with its antiplatelet activities and none of the detected compounds were reported to have antiplatelet activities. It is shown from this research, that the compounds which were identified in the methanol extracts of shallot, including propanethion, methyl propyl disulfide, dipropyl disulfide and methyl propyl trisulfide, especially methyl propyl disulfide, were more enriched in the most potent variety (Local Kupang) compared to the least active variety (Kuning Gombong) (Tabel 3).

Conclusion

The methanol extracts of garlic, shallot, chive, yellow onion, and green onion showed inhibition to platelet aggregation, garlic extracts being the most potent. Antiplatelet aggregation activities in extracts of garlic and shallot grown in Indonesia were dependent on the growth area and varieties. There tended to be positive correlations between the amount of volatile reducing compounds, especially the organosulfur compounds, and the antithrombotic activities in extracts.

As garlic showed the highest activity, it is considered having the greatest potencies to be further evaluated as raw material to produce functional food ingredients to prevent coronary heart disease. Especially in Indonesia, however, shallot seems to have potency to be used as substitute for garlic. Although its activity was not as high as garlic, shallot are abundantly produced in Indonesia due to its easy cultivation (Biro Statistika, 1993) and it is also consumed by most population in much greater quantities than garlic.

Acknowledgement

Financial support was obtained from DP_3M, ADAET-Indonesian Ministry of Education. We thank Dr. Rahayuningsih Dharma DSPK and staffs, Cipto Mangunkusumo Hospital, for laboratory supporting, and Dr. Kirk L. Parkin for editorial suggestions on the manuscript. We also thank to Mr. Agung P. and Arief H. for technical assistance.

References

Apitz-Castro, R., Cabrera, S., Cruz, M. R., Ledezman, E., and Jain M. K., Effect of garlic extract and three pure component isolated from it in human platelet aggregation, arachidonate metabolism, release reaction and platelet ultrastructure. *Thrombosis Research* **32**, 155-169 (1983).

Apriyantono, A., Fardiaz, D., Puspitasari, N. L., Yasni, S., and Budijanto, S., *Petunjuk Laboratorium Analisa Pangan* (Food analysis manual). PAU-Pangan dan Gizi, IPB, Bogor (1989).

Ariga, T., Oshiba, S., and Yamada, T., Platelet aggregation inhibitor in garlic. *Lancet* **1**, 150-151 (1981).

Biro Pusat Statistik., *Statitistik Indonesia Tahun* 1992 (Indonesian statistical data in 1992). PPS, Jakarta (1993).

Block, E., The chemistry of garlic and onion. *Scientific American*, March, 114-119 (1985).

Block, E., Correspondence : Flavor artifacts. *J. Agric. Food Chem.* **41**, 592 (1993).

Block, E., Ahmad, S., Catalfamo, J. L., Jain, M. K. and Apitz-Castro, R., Antithrombotic organosulfur compounds from garlic: strutural, mechanistic, and synthentic studies. *J. Am. Chem. Soc.* **108**, 7045-7055 (1986).

Bordia, A., Effect of garlic on human platelet aggregation *in vitro*. *Atherosclerosis* **30**, 355-360 (1978).

Bordia, A., Bansal, H. C., Arora, S. K., and Singh, S. V., Effect the essential oils of garlic and onion on alimentary hyperlipemia. *Atherosclerosis* **21**, 15-19, (1975).

Farbor, G. R. and Hanley, A. B., The genus Allium. Part 2. *CRG Crit. Rev. Food Sci. Nutr.* **22**, 273-377 (1967).

Freeman, G. G. and Whenham, R. J., A survey of volatile components of some *Allium* species in term of S-alk(en)yl-L-cysteine sulphoxides present as flavor precursors. *J. Sci. Food Agric.* **26**, 1869-1886 (1975).

Iberl, B., Winkler, G., Muller, B., and Knobloch. K. , Quantative determination of allicin from garlic by HPLC. *Planta Med.* **56**, 320-326 (1990).

Kyriakides, M. L., Relation between the structure of Alliin analogues and their inhibitory effect on platelet aggregation. *Phytochemistry* **24**(7), 1593-1594, (1985).

Morimitsu, Y. and Kawakishi, S., Inhibitors of platelet aggregation from onion. *Phytochemistry* **24**(7), 1593-1594, (1990).

Morimitsu, Y., Morioko, Y. and Kawakishi, S., Inhibitors on platelet aggregation generated from the mixtures of Allium species and/or S-(alk(en) yl-L-cysteine sulfoxides. *J. Agric. Food Chem.* **40**, 368-372, (1992).

Nishimura, H., Wijaya, C. H., and Mizutani, J., Volatile flavor components and antithrombotic agents: Vinyldithiin from *Allium victorialis*. *J. Agric. Food Chem.* **36**, 563-566, (1988).

Ochse, J. J., and Van den Brick, R. C. B. Vegetable of the Dutch East Indiest. Archipel Drukkerij Butenzorg, Java, 1931, pp.55.

Tokoro, I., Kinosei shokuhin (Functional food). *Takasago koryo jiho* (in Japanese) 97-25, (1989).

Whitaker, J. R., Development of flavor, odor and pungency in onion and garlic, E. M. Mrak and G. F. Stewart (eds.), *Advances in Food Research*, Vol 22, C.O. Chihchester. Academic Press, New York. 1976.

Wijaya, C. H., Nishimura, H., Takana, T., and Mizutani, J., Influence of drying methods on volatile sulfur constituents of caucas. *J. Food Sci.* **1**, 72-75, (1991).

Wijaya, C. H., Muchtadi, D., and Prihambodo, A., Pemanfaatan potensi bawang-bawangan sebagai "health food supplements" : pengujian in vitro aktifitas antitrombotik lima varietas bawang merah (*Allium cepa* var. aggregatum). *Bul. Teknol. Industri Pangan* 5(2),15-21. (in Indonesia), (1994).

Wu, J. L., Chou, C. C., Chen, M. M., and Wu, M. C., Volatile flavor compounds from shallot. *J. Food Sci.* **47**, 606-608, (1982).

Yu, T. H. and Wu, C. M., Effects of pH on the formation of flavor compounds of dirupted garlic. *J. Chromatography* **462**, 137-145, (1989).

(Accepted May 14, 1996)