BULLETIN OF TAIPEI MEDICAL COLLEGE, VOL. 16, JUNE 1987
P. 139-147

Hemagglutination Activities and Hemolysing Activities of Formosan Plant Extracts

CHUNG-FANG CHEN, CHIEN-TSU CHEN, CHEH-MIN SU and
YIH-CHIH TUNG

SUMMARY

43 species of seeds, nuts or beans in Taiwan were examined for hemaggultination or hemolysing activity. The plant extracts were prepared by extraction with 5% acetic acid then lyophilized after neutralization and dialysis. It was found that the extracts from 17 species could agglutinate the erythrocytes of human, rat or sheep: Nut extracts from betel palm, Chinese fan palm and bamboo palm, as well as seed extracts of rose-apple, egg-fruit, and avocado all had high hemagglutination activities toward the erythrocytes of human, rat and sheep. For extracts that cause RBC lysis among 5 species, which camellia was found with the highest hemolysing activity. In disc electrophoresis using pH 8.9-7.5% gel system, the components in all extracts rapidly migrated to the anode and formed a single or major band which could be stained with Coomassie blue or toluidine blue. Contents of carbohydrate, uronic acid and protein from the extracts were determined. The results indicated that protein and carbohydrate of the glycosaminoglycan type made up the extract components. The protein content was determined by Bradford's method and Lowry's method. It was later discovered that Lowry's method was not suitable for plant seeds; overestimation of the protein content by Lowry's method was especially noted in those seed extracts with hemagglutination activity.

Key words: Hemagglutination, Hemolysis, Formosan Plants, Disc electrophoresis, Glycosaminoglycan.

A number of lectins or hemagglutinins had been purified from the plants[1]. These lectins of plant origin, owing to their biological activities, had been widely used as research tools in fields of mitogenic action, cancer therapy and cell menbrane structure[2]. From 111 native plant species in Taiwan the lectin content in saline seed extracts had been estimated by Wang and Liu[3]. Recently, we discovered that 5%

Department of Biochemistry, Taipei Medical College
Received for Publication: February 5, 1987.

acetic acid extracts of the seeds of longan and litchi had high hemagglutination activities toward the erythrocytes of different animal sources[4]. Therefore, we used the same extraction procedure to examine 43 plant species in Taiwan. The extracts of 17 species had hemagglutination activity whereas 5 species had hemolytic activity. Disc electrophoresis of the extracts with hemagglutination or hemolysing activity had been carried out, and the carbohydrate/protein contents of the extracts were determined.

MATERIALS AND METHODS

The plant samples used during our study were bought from seed stores, fruit and vegetable markets, or harvested from the plants by ourselves. The samples were dried under sunshine then ground in a high speed electric grinder before use.

Preparation of the plant extracts:

Twenty gm of a powdered plant sample was extracted with 200 ml 5% acetic acid solution in a mixer for five minutes, and filtered through a Whatman No. 1 paper with suction. The filtrate was adjusted to pH 7 by dropwise addition of conc. NaOH solution and filtered upon precipitate formation. The extract was dialyzed against distilled water and lyophilized.

Determination of hemagglutination titer or hemolysing titer:

The erythrocytes of human, albino rat or sheep were collected and washed with phosphate buffered saline pH 7.2 (PBS) just before use. 0.1 ml two-fold dilutions of a sample under test in PBS and 0.1 ml 1% erythrocyte suspension in PBS were mixed and incubated at 37°C for one hour in a U-shape well microtiter plate (Cook engineering company). The Hemagglutination titer or hemolysing titer was expressed as the reciprocal of the minimal concentration (mg/ml) of the sample that induce hemagglutination or hemolysis. The samples which could not induce hemagglutination or hemolysis at 1 mg/ml were considered to have nil activity.

Carbohydrate content was determined by reaction with anthrone using glucose as standard[5]. Uronic acid was determined by carbazole method using glucuronolactone as standard[6]. Protein content was estimated by the methods of Bradford[7] and Lowry[8], with Bovine serum albumin as standard.

Disc electrophoresis of 0.2 mg plant extracts was carried out in tubes containing pH 8.9-7.5% polyacrylamide gel. Bromophenol blue was used as a dye marker while 5 mA current was applied to each tube. The gels were stained with 0.25% Coomassie blue in 7% acetic acid or with 0.02% toluidine blue in 0.6% acetic acid and destained in 7% or 3% acetic acid.

RESULTS AND DISCUSSION

The extracts from 17 plant species with hemagglutination activity are given in Table 1. Note that the extracts of betal palm, Chinese fan palm, bamboo palm, rose-apple, egg-fruit, and avocado have high

Table 1. Hemagglutination Activities of the Plant Extracts

Scientific name (Family)	Common name (Part used)	Hemagglutination titer					
		Rat	Sheep	Human			
				O	A	B	AB
Areca Catechu (Palmae)	Betel palm (nut)	512	256	512	512	512	512
Livistonia chinensis (Palmae)	Chinese fan palm (nut)	256	256	512	512	512	512
Chrysalidocarpus lutescens (Palmae)	Bamboo palm (nut)	256	512	512	512	256	512
Phoenix formosana (Palmae)	Formosan date palm (nut)	2	0	0	0	0	0
Syzygium jambos (Myrtaceae)	Rose-apple (seed)	2048	512	512	256	256	256
Lucuma nervosa (Sapotaceae)	Egg-fruit (seed)	256	256	256	256	256	256
Persea spp (Lauraceae)	Avocado (seed)	128	128	128	128	128	256
Eleocharis dulcis (Cyperaceae)	Water chestnut (corms)	16	32	4	4	32	8
Dolichos Lablab (Leguminosae)	Mauntain ebony (bean)	0	0	64	128	64	128
Bauhinia purpurea (Leguminosae)	Hyacinth (bean)	0	0	0	0	0	4
Eriobotrya japonica (Rosaceae)	Loquat (seed)	16	16	0	0	4	4
Solanum tuberosum (Solanaceae)	Potato (tubers)	8	0	8	4	32	4
Alpinia formosana (Zingiberaceae)	Taiwan alpinia (seed)	4	32	4	4	4	4
Colocasia antiquorum (Araceae)	Taro (corms)	64	0	0	0	0	4
Nelumbo nucifera (Nymphaeaceae)	Lotus seed (seed)	2	0	0	0	0	0
Ganoderma lucidum (Polyporaceae)	Lirngjy (fruit body)	4	0	8	0	2	2
Castanea crenata (Fagaceae)	Chestnut (nut)	2	0	0	0	0	0

agglutination activities toward the human, rat, and sheep RBC. The results implicate that the nuts of palms are good sources of material for the investigation of phyto-hemagglutinins, especially in a subtropical location such as Taiwan. Although mauntain ebony extract could not agglutinate rat and sheep erythrocytes, their harbor high agglutination titers toward different types of human erythrocytes. Five extracts causing hemolysis is shown in Table 2. Among the five species, camellia has the highest hemolysing titer. The plant extracts which could not agglutinate or lyse the erythrocytes are listed in Table 3.

Disc electrophoretic patterns of the plant extracts with hemagglutination or hemolysing activity are shown in Fig. 1 and Fig. 2. At pH 8.9, the components in all extracts migrated rapidly to the anode,

Table 2. Hemolysing Activities of the Plant Extracts

Scientific name (Family)	Common name (Part used)	Hemolysing titer					
		Rat	Sheep	Human			
				O	A	B	AB
Camellia japonica (Theaceae)	Camellia (seed)	2048	2048	1024	2048	1024	4096
Thea sinensis (Theaceae)	Tea (seed)	64	8	32	32	64	32
Luffa cylindrica (Cucurbitaceae)	Loofah gourd (seed)	16	8	16	8	8	16
Momordica Charantia (Cucurbitaceae)	Bitter gourd (seed)	16	4	16	16	8	8
Ipomoea aquatica (Convolvulaceae)	Water convolvulus (seed)	2	0	16	8	8	8

Table 3. Plant Extracts Have No Hemagglutination and Hemolysing Activity

Family	Scientific name	Part used
Anacardiaceae	Anacardium occidentale	seed
Annonaceae	Annona squamosa	seed
Apocynaceae	Thevetia peruviana	seed
Caricaceae	Carica papaya	seed
Chenopodiaceae	Spinacia oleracea	seed
Cruciferae	Raphanus sativus	seed
Cucurbitaceae	Benincasa hispida	seed
	Citrullus vulgaris	seed
	cucumis sativus	seed
	Cucurbita moschata	seed
Gramineae	Hordeum vulgare	grain
	Sorghum bicolor	grain
	Triticum aestivum	grain
	Zea mays	grain
Leguminosae	Delonix regia	bean
	Glycine max (black bean)	bean
Pinaceae	Pinus palustris	seed
Rutaceae	Citrus reticulata	seed
	Citrus sinensis	seed
	Murraya paniculata	seed
Sterculiaceae	Sterculia nobilis	seed

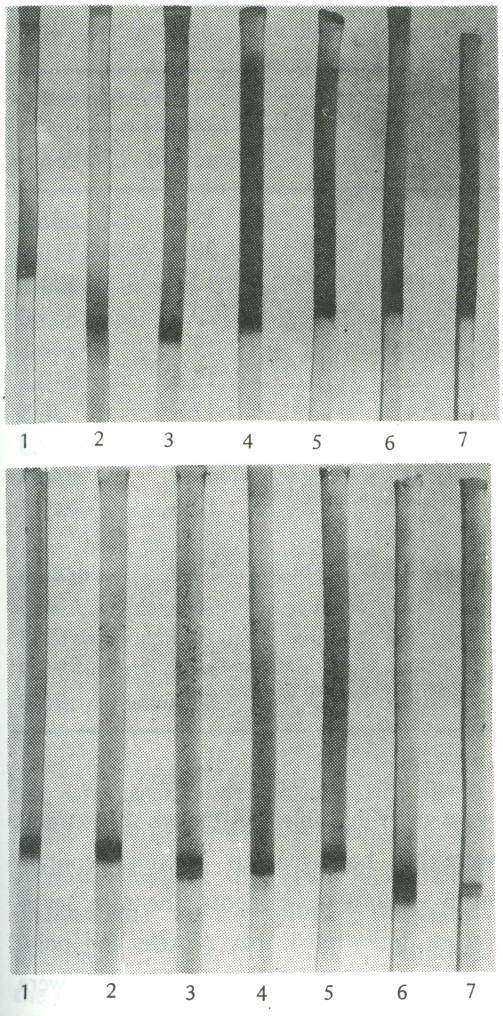
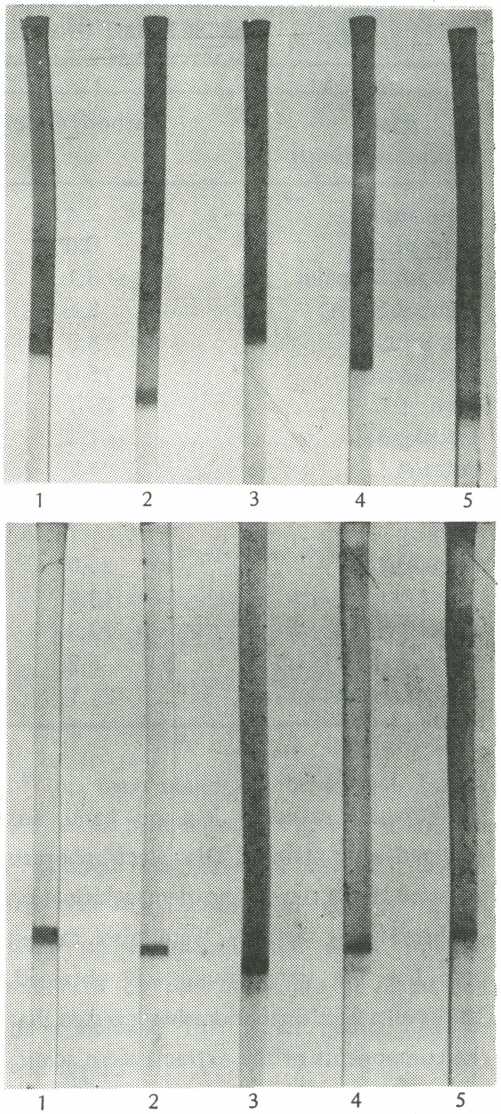


Fig.1. Disc electrophoresis in pH 8.9-7.5% polyacrylamide gel of the plant extracts with hemagglutination activity. 1. litchi, 2. rose-apple, 3. egg-fruit, 4. avocado, 5. bamboo palm, 6. betel palm, 7. Chinese fan palm. Upper: stained with Coomassie blue; Lower: stained with toluidine blue.



Fgi. 2. Disc electrophoresis in pH 8.9-7.5% polyacrylamide gel of the plant extracts with hemolysing activity. 1. camellia, 2. loofah gourd, 3. water convovulus, 4. tea, 5. bitter gourd. Upper: stained with Coomassie blue; Lower: stained with toluidine blue.

and formed a single or major band that almost coincided with the position of the marker dye. Litchi extract had many components which could be separated by ammonium sulfate precipitation or column chromatography[4], but only one band was observed after electrophoresis. The crude extract of longan also showed only one band after electrophoresis in the same gel system[9], nevertheless, the extract could be separated into five fractions by pH adjustment. In electrophoresis using pH 4.3-7.5% gel system, the components of the extracts did not migrate into the

Table 4. Carbohydrate, Uronic Acid and Protein Contents (Percentage) of the Plant Extracts

Plant	Carbohydrate	Uronic acid	Protein	
			Bradford	Lowry
Rose-apple	7.4	1.6	13.4	635.8
Betel palm	0.0	1.8	20.5	712.9
Chinese fan palm	3.1	1.6	17.3	571.0
Bamboo palm	8.6	1.2	20.9	539.0
Egg-fruit	8.3	1.0	19.3	248.3
Avocado	5.0	2.1	15.5	612.5
Taro	20.7	4.9	17.8	36.6
Litchi	7.4	0.6	4.2	69.3
Potato	1.7	0.7	49.2	50.7
Taiwan alpinia	21.1	18.2	10.6	159.6
Camellia	33.0	9.7	24.0	23.4
Tea	13.2	3.9	46.3	62.8
Loofah gourd	12.9	5.4	6.5	54.6
Bitter gourd	6.2	4.1	36.9	95.9
Water convolvulus	21.1	4.2	15.8	26.5

gel (toward cathode) and no band was detected after staining. Disc electrophoresis of some extracts with no hemagglutination or hemolysing activity were also carried out, but the same result was obtained. The results of electrophoresis suggested that the components of the extracts were acidic, since they could move rapidly to the anode at pH 8.9 but would not migrate to the cathode at pH 4.3.

After electrophoresis, the band in the gel could be stained with Coomassie blue or toluidine blue. The blue colored band stained with toluidine blue gradually changed to green and faded. This fact suggested that glycosaminoglycan (mucopolysaccharide) or proteoglycan might be the component of the extracts. The contents of carbohydrate, uronic acid and protein were given in Table 4. Although the extracts were not homogeneous and may contain many various components, the data nevertheless interpreted that protein and polysaccharide of glycosaminoglycan type were the major components of the extracts. The results of protein content indicated that Lowry's method was not suitable for plant seeds. Upon inspection of the values obtained by Lowry's method, we found that overestimation of the protein content was especially noted in the extracts with hemagglutination activity. Therefore, we calculated the ratio of the protein contents determined by the two methods (Lowry/Bradford) and compared with the hemagglutination titer against rat erythrocytes (Table 5). The values of the ratio of extracts with hemagglutination activity were higher than

Table 5. Relationship between the Hemagglutination Titer against Rat Erythrocytes and the Ratio of the Protein Contents Determined by the Methods of Lowry and Bradford

Plant	Hemagglutination titer	Ratio (Lowry/Bradford)
Rose-apple	2048	47.5
Betel palm	512	34.8
Chinese fan palm	256	33.0
Bamboo palm	256	25.8
Egg-fruit	256	12.9
Avocado	128	39.5
Taro	64	2.1
Litchi	32	16.5
Potato	8	1.0
Taiwan alpinia	4	15.1
Camellia		1.0
Tea		1.4
Loofah gourd		8.4
Bitter gourd		2.6
Water convolvulus		1.7

ten, whereas ratio for extracts with hemolysing activity were lower than ten. The proportionality of the ratio and hemagglutination titer was also found in the extract of longan and its fractions[9]. Although the extracts of taro and potato could agglutinate the erythrocytes, the values of the ratio were very low. We should note, however, that the parts of these two plants being used were corms and tubers but not seeds.

Seeds of bitter gourd had hemagglutination activity and a form of lectin had been purified from the seeds of this plant[10]. The seed extract from bitter gourd had hemolytic properties rather than hemagglutination. Although the phytohemagglutinin of red kidney bean (*Phaseolus vulgarid*) had been purified and widely used[1], we found that the hemagglutinin of this bean could not be extracted with 5% acetic acid solution. In our study, we discovered that extracts from 21 species had no hemagglutination activity. Since some phytohemagglutinins could not be extracted by our procedure, it could not be concluded that other plants had no hemagglutinin.

REFERENCES

1. GINSBURG V: Methods in Enzymology Vol. XXVIII, Complex Carbohydrates Part B, New York and London, Academic Press, p. 313-368, 1972.
2. LIS H, SHARON N; The Biochemistry of lectins (Phytohemagglutinins). Annu Rev Biochem 42; 541-574, 1973.
3. WANG CY, LIU CF: Studies of

Native Plants in Taiwan for Lectin Content. Chinese J Microbiol 8; 230-235, 1975.

4. TUNG YC, CHEN CT, SU CM: Hemagglutination Activities of the Seed Extracts of *Litchi chinensis* and *Euphoria Longana*. Bull Taipei Med College 15; 119-126, 1986.
5. NEUFELD EF, GINSBURG V: Methods in Enzymology Vol. VIII, Complex Carbohydrates, New York and London, Academic Press, p. 4, 1966.
6. BITTER T, MUIR HM: A Modified Uronic Acid Carbarzole Reaction. Anal Biochem 4; 330-334, 1962.
7. BRADFORD M: A Rapid and Sensitive Method for Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding. Anal Biochem 72; 248-251, 1976.
8. LOWRY OH, ROSEBROUGH NJ, FARR AL, RANDALL RJ: Protein Measurement with the Folin Phenol Reagent. J Biol Chem 193; 265-275, 1951.
9. CHEN CT, SU CM, TUNG YC: Studies on the Hemagglutinins: Fractionation and Chemical Characterization of the Acid Extract. Bull Taipei Med College 16; 139-146, 1987.
10. MAZUMDER T, GAUR N, SUROLIA A: The Physicochemical Properties of the Galactose-Specific Lectin from *Momordica charantia*. Eur J Biochem 113; 463-470, 1981.

台灣植物抽取物之紅血球凝集活性及溶血活性

陳瓊芳　陳建志　蘇哲民　董一致

採集 43 種台灣植物的種子，核或豆類，研究其紅血球凝集活性或溶血活性。以 5 %醋酸水溶液抽取植物成分，抽取液經中和且透析後，冷凍乾燥取得植物抽取物。 17 種抽取物能使人，鼠，和羊的紅血球凝集。其中核抽取物，如檳榔、蒲葵、及黃椰子。和種子抽取物，如蒲桃、仙桃、酪梨，對於人、鼠及羊的紅血球具有高的紅血球凝集活性。另 5 種抽取物會使紅血球溶血，其中苦茶有相當高的溶血作用。在 pH 8.9— 7.5 %的膠體系統中做電泳，所有抽取物的成分都快速向正極移動，且形成單一或主要的環帶，可致 Comassie blue 及 Toluidine blue 染色，荔枝與龍眼抽取物含有許多成分，但其粗抽取物在此系統中，也是呈一條環帶且所有抽取物在 pH 4.3— 7.5 %膠體系統中，不會向陰極移動，經染色後未見環帶。所以推測抽取物的成分是酸性。另又做抽取物的醣類，Uronic acid 及蛋白質的含量測定，以 Bradford 及 Lowry 方法測定蛋白質，由其結果發現具有凝集活性的種子抽取物，以 Lowry 方法測得的蛋白質含量都偏高，此方法不適合植物的種子抽取物。由實驗知道抽取物不是單一成分，且可能含有許多成分。推測抽取物的成分是蛋白質及 glycosaminoglycan 型的多醣類。在此研究中，有 21 種抽取物沒有凝集作用，其中菜豆（Phaseolus vulgaris）[1] 的凝集素已被純化且廣泛的使用，但以 5 %醋酸水溶液無法抽得其凝集素，所以有些植物凝集素不能用我們的方法抽取，並不表示這些植物沒有植物凝集素。

台北醫學院生化學科

民國七十六年二月五日受理