Original article

Anti-H lectin *Momordica charantia* blood grouping reagent produced by the National Blood Centre, Thai Red Cross Society

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Abstract:

Introduction: *The National Blood Centre, Thai Red Cross Society, previously produced anti-H lectin from Ulex europaeus seeds from 1972 to 2013, and now produces anti-H murine monoclonal antibodies (MoAb). However, these antibodies cannot detect H substances in saliva, so the results of the hemagglutination inhibition test (HAI) are incorrect. We studied local plant seeds for anti-H lectin activities, such as bitter gourd (Momordica charantia) seed, which can be purchased at an agricultural supply store.* ***Objective:*** *To explore the potential seeds for replacing Ulex europaeus to use as a source for anti-H lectin reagents production.* ***Materials and Methods:*** *Seventy-five plant seeds were extracted for anti-H lectins by saline extraction. Plant seeds-extracted supernatants were tested with group A, B, O, and AB red blood cells (RBCs) by the standard tube test to detect agglutination and hemolysis reaction, and then continued testing with Ahm, Bhm, and Ohm RBC and selected those with no reactions that showed anti-H activities. There are six seed extracts from four varieties of Momordica charantia (Cyber, Moddam, Okinawa, and Chinese bitter melon) and two varieties of Psophocarpus tetragonolobus (Green and Purple winged bean). The extracts from the selected plant seeds were further tested for titration with A, B, O, AB, Ahm, Bhm, and Ohm RBCs and saliva HAI, adsorption, heat elution, specificity, and stability.* ***Results:*** *The extracts of every anti-H lectin from Momordica charantia caused hemolysis. The suitable dilution of anti-H lectin Momordica charantia was Cyber titer 1:64, Moddam titer 1:16, and Okinawa and Chinese bitter melon titer 1:32. The HAI test for H substances in saliva revealed that anti-H lectins Momordica charantia varieties Cyber and Okinawa showed the same results as anti-H lectin Ulex europaeus in all tested saliva samples. The observed specificity of each anti-H lectin corresponded to the theory. Anti-H lectins from Momordica charantia, Cyber, and Okinawa showed specificity to the H antigen. The stability study showed that anti-H lectins from Momordica charantia Cyber and Okinawa remained stable for 18 months, but the 18th-month Cyber sample was more potent than the Okinawa sample. Adsorption and heat elution showed that six plant seed extracts are truly anti-H.* ***Conclusion:*** *Momordica charantia Cyber is the most suitable source for producing anti-H lectin reagents for use in blood bank laboratories due to its high titration and good stability.*

Keywords : ● *Anti-H* ● *Ulex europaeus* ● *Momordica charantia* ● *Lectin* ● *H substances*

J Hematol Transfus Med. 2026;36:7-19.

Received 24 September 2025 Corrected 29 October 2025 Accepted 21 November 2025

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นิพนธ์ต้นฉบับ
น้ำยาตรวจหมู่โลหิตแอนติเอชเลคติน *Momordica charantia* ผลิตโดยศูนย์บริการโลหิตแห่งชาติ สภากาชาดไทย

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บทคัดย่อ

บทนำ *ศูนย์บริการโลหิตแห่งชาติ สภากาชาดไทย ผลิตแอนติเอชเลคตินจากเมล็ด Ulex europaeus ตั้งแต่ปี ค.ศ. 1972 จนถึงปี ค.ศ. 2013 ปัจจุบันเปลี่ยนเป็นการผลิตแอนติเอชมิวรีนโมโนโคลนัล ซึ่งพบปัญหาเนื่องจากไม่สามารถตรวจสาร H ในน้ำลาย ดังนั้น ผลยับยั้งการจับกลุ่มของเม็ดเลือดแดง (HAI) จึงไม่ถูกต้อง จากการนำเมล็ดเมล็ดพืชท้องถิ่นชนิดอื่น ๆ มาศึกษาหาแอนติเอชแลคติน พบว่า เมล็ดมะระขี้นก (Momordica charantia) มีแอนติเอชเลคติน ซึ่งเป็นเมล็ดพันธุ์ที่สามารถหาซื้อได้ตามร้านขายอุปกรณ์ทางการเกษตร* ***วัตถุประสงค์*** *เพื่อหาเมล็ดพืชที่เหมาะสมมาทดแทนเมล็ด Ulex europaeus สำหรับใช้ในการผลิตแอนติเอชเลคติน* ***วัสดุและวิธีการ*** *นำเมล็ดพืชท้องถิ่นเจ็ดสิบห้าชนิดมาสกัดแอนติเอชเลคตินด้วยน้ำเกลือ ทดสอบน้ำสกัดจากเมล็ดพืชกับเซลล์เม็ดเลือดแดงหมู่ A, B, O และ AB ด้วยวิธีมาตรฐานหลอดทดลอง คัดเลือกน้ำสกัดจากเมล็ดพืชที่เกิดปฏิกิริยาจับกลุ่มของเม็ดเลือดแดงและทำให้เม็ดเลือดแดงแตก มาทดสอบต่อกับเซลล์เม็ดเลือดแดง Ahm, Bhm และ Ohm ถ้าน้ำสกัดจากเมล็ดพืชชนิดใดไม่เกิดปฏิกิริยาดังกล่าว แสดงว่ามีแอนติเอช พบว่าสารสกัดจากเมล็ดพืชหกชนิดประกอบด้วย Momordica charantia สี่สายพันธุ์ ได้แก่ ไซเบอร์ มดดำ โอกินาว่า มะระจีน และ ถั่วพู (Psophocarpus tetragonolobus) สองสายพันธุ์ ได้แก่ ฝักเขียวและฝักม่วง เลือกสารสกัดจากเมล็ดพืชดังกล่าวมาทดสอบหาความแรงกับเซลล์เม็ดเลือดแดง A, B, O, AB, Ahm, Bhm และ Ohm ทดสอบกับน้ำลาย (HAI) ดูดซับและอีลูชั่นด้วยความร้อน ตรวจหาความจำเพาะและความคงทน* ***ผลการศึกษา*** *น้ำสกัดแอนติเอชเลคติน Momordica charantia ทุกชนิดทำให้เม็ดเลือดแดงแตก น้ำสกัดเจือจางของแอนติเอช Momordica charantia ซึ่งมีความแรงที่เหมาะสม คือ ไซเบอร์ความแรง 1:64 มดดำความแรง 1:16 โอกินาว่าและมะระจีนความแรง 1:32 ผลทดสอบ HAI ของสาร H ในน้ำลาย พบว่าแอนติเอชเลคติน Momordica charantia ไซเบอร์และโอกินาว่า มีผลตรงกับแอนติเอชเลคติน Ulex europaeus ทุกตัวอย่าง แอนติเอชเลคตินทุกชนิดมีความจำเพาะเป็นไปตามทฤษฎี แอนติเอช Momordica charantia ไซเบอร์และโอกินาว่า บ่งชี้ถึงความจำเพาะเจาะจงกับแอนติเจน H การศึกษาความคงทนแสดงให้เห็นว่าแอนติเอชเลคติน Momordica charantia ไซเบอร์และโอกินาว่า มีความคงทนสำหรับ 18 เดือน แต่เดือนที่ 18 ตัวอย่างไซเบอร์มีความแรงมากกว่าตัวอย่างโอกินาว่า การดูดซับและอีลูชั่นด้วยความร้อนแสดงให้เห็นว่าน้ำสกัดจากเมล็ดพืชทั้งหกชนิดเป็นแอนติเอชจริง* ***สรุป*** *Momordica charantia ไซเบอร์ เหมาะสมที่สุดที่จะนำมาผลิตเป็นน้ำยาแอนติเอชเลคตินสำหรับใช้ในห้องปฏิบัติการธนาคารเลือดแทนการผลิตจาก Ulex europaeus เนื่องจากมีความแรงสูงและมีความคงทนดี*

คำสำคัญ : ● *แอนตี้เอช* ● *ยูเร็ก ยูโรเปียส* ● *มะระขี้นก* ● *เลคติน* ● *สารเอช*
วารสารโลหิตวิทยาและเวชศาสตร์บริการโลหิต. 2569;36:7-19.

Introduction

Many plant seeds contain carbohydrate-binding proteins called lectins that are found in corals and beans, fungi, bacteria, and animals. Lectins are proteins or glycoproteins that are of non-immune origin and can combine carbohydrate molecules or carbohydrate groups in glycoproteins or glycolipid complexes. Carbohydrate blood group antigens on the red blood cells (RBCs) membrane surface can be detected by a lectin specific to these antigens. Plant seed extracts were a highly popular research topic in the fifth decade of the 20th century, and many researchers published studies on blood group-specific activity. The important reports on seed extracts specific to blood groups are Renkonen in Helsinki (1948), Boyd and Reguera in Boston (1949), Bird in India (1955), and Boyd in Egypt (1950). *Vicia cracca* contains anti-A, the first report of a blood-group specificity that differentiates between blood group A_1 and A_2, as shown by Koulumies (1949). *Dolichos biflorus* seeds exhibit anti-A_1 activity reported by Bird (1952). The seeds of *Phaseolus coccineus* present specific anti-A_1 activity, and *Falcata japonica* present specific A_2 antigens. The seed coats of *Evonymus* species, the fungi *Fomes fomentarius*, *Clavulinopsis fusiformis*, and the seaweed *Ptilota plumose* exhibit anti-B activity. Renkonen first detected anti-H activity in seed extracts of *Cystisus sessilifolius*, *Laburnum alpinum*, and *Psophocarpus tetragonolobus*. Several seed lectin extracts contain anti-H, but very few of them are used as anti-H reagents in blood bank laboratories, except lectin extracted from *Ulex europaeus* seeds, which are the most popularly used as anti-H lectin reagents.[1] Anti-H is necessary in blood bank laboratories because persons who have the Bombay (O_h) phenotype do not express the H antigen on their RBCs and usually contain naturally occurring anti-H. This is a problem in blood transfusion because if a Bombay patient's phenotype is transfused with blood that has the H antigen on RBCs, an acute hemolytic transfusion reaction occurs due to anti-H in plasma, so Bombay (O_h) patients must receive blood only from other Bombay (O_h) phenotype blood donors. The H antigen-soluble substances are translated by *FUT1(H)* and *FUT2(Se)* genes, on 19q13.3.1. The *H* gene encodes for fucosyltransferase. A point mutation inactivates the gene, leading to both the Bombay and para-Bombay phenotypes. If both *H*-gene linkages are inactivated (*hh*), fucosyltransferase cannot be translated, so no H antigen on RBCs and no H substances in their plasma.[2]

In the past, the Antiserum and Standard Cells Production Section of the National Blood Centre, Thai Red Cross Society, produced anti-H lectins from *Ulex europaeus* seeds purchased from Europe. We can no longer buy *Ulex europaeus* seeds, so we now produce anti-H murine MoAb instead. However, another problem occurs because the anti-H murine MoAb cannot detect H substances in saliva.[2] Because *Ulex europaeus* is a European native plant that cannot be grown in tropical areas, we try to find another local seed plant to produce anti-H lectin. *Momordica charantia* seeds are well known for containing anti-H lectins and are a common ingredient in Thai cuisine. Farmers widely cultivate this crop. Their cultivation seeds can be purchased at agricultural supply stores, making suitable seed for anti-H lectin production.

Materials and Methods

This study was approved by the Research Ethics Committee, National Blood Centre, Thai Red Cross Society (COA No. NBC 10/2022).

Materials

1. Seventy-five plant seeds were extracted for anti-H lectin. There is a list of sixty-three scientific names. Some plant seeds have different common names but the same scientific name, as follows: *Solanum tuberrosum*, *Cumis sativa*, *Carica papyya*, *Cucurbita pepo var. cylindica*, *Cucumis melo*, *Moringa oleifera*, *Luffa acutangular*, *Lactuca sativa, Coccinia grandis*, *Mirabilis jalapa*, *Zinnia angustifolia Kunth*, *Cosmos sulphureus*, *Thumbergia alata*, *Symphyotrichum ericoides*, *Cucurbita*

moschata, *Momordica charantia*, *Trichosanthes anguina*, *Vigna unguiculata subsp. sesquipedalis*, *Brassica oleracea var. gongylodes*, *Basella alba*, *Pisum sativum*, *Sesbania javanica miq*, *Psophocarpus tetragonolobus*, *Crotalaria juncca*, *Viga unguiculata walp*, *Luffa acutangular*, *Cucumis melo*, *Phaseolus vulgaris*, *Cucumis sativus*, *Citrullus lanatus*, *Lopomoea alba*, *Vigna unguiculata*, *Raphanus sativus var. caudatus Alef*, *Citrullus lanatus*, *Carca papaya*, *Luffa cylindrica*, *Lagenaria siceraria Standl*, *Cucurbita pepo*, *Rothmannia witti*, *Brassica juncea*, *Ocimum basilicum f. citratum Back*, *Prunus mume*, *Prunus cerasoides*, *Cajanus cajan*, *Plukenetia volubilis*, *Dionaea muscipula*, *Mucuna pruriens var. utilis*, *Murraya siamensis Craib*, *Cassia fistula*, *Gloriosa superba*, *Tecama stans*, *Nelumbo nucifera*, *Gomphrena globasa*, *Solanum molongena*, *Solanum torvum*, *Raphanus sativus var. Longipinnatus*, *Hibiscus sabdariffa*, *Pachyrhizus erosus*, *Daucus carota subsp. Sativa Thell*, *Clitoria ternatea*, *Portulaca grandiflora*, *Lagenaria siceraria (Mol) Standl*

2. The inactivated frozen saliva of 206 samples from blood donors at the National Blood Centre, Thai Red Cross Society, remaining from the article ABH secretor status in the Thai population were used.[3]
3. 0.9% Normal saline solution (NSS)
4. 4% Bovine serum albumin
5. A, B, O, AB, A_2, A_3, B_3, A_2B, A_3B, A_1B_3, Ohm, Ahm, Bhm RBCs samples from blood donors at the National Blood Centre, Thai Red Cross Society, and cord blood from commercial.

Methods

Plant seeds, saline extraction method, and antibody titration[4]

Place 10 grams of seeds in a blender until the particles resemble coarse sand, then grind to a fine powder in a large test tube or a small beaker. Add three to four times their volume of 0.9% NSS, then soak overnight. Thereafter, the supernatants were centrifuged at 3,000 rpm for 5 minutes, and the supernatants were separated. Plant seeds extracted supernatants were tested with group A, B, O, and AB RBCs by the standard tube test method, incubated at room temperature (RT) for 15 minutes, and read for agglutination and hemolysis reactions. The chosen plant seed extract produced supernatants were those that elicited agglutination and hemolysis reactions. Continued testing with Ahm, Bhm, and Ohm RBCs; all had no agglutination and hemolysis. The plant seed-extracted supernatants that showed hemolysis should be appropriately diluted before retesting with A, B, O, and AB RBCs until no hemolysis is detected. If agglutination occurs, proceed to test with Ahm, Bhm, and Ohm RBCs; no reaction must occur. Seeds capable of producing anti-H lectins were titrated again with A, B, O, AB, Ahm, Bhm, and Ohm RBCs to determine the appropriate dilution.

Hemagglutination inhibition test (HAI)[3,4]

The optimal dilution of anti-H lectins was determined by performing a 2-fold dilution series in NSS and testing with the corresponding standard O cells. Then selected the dilutions that gave 2+ (score 8) agglutination results as follows: no. 1 *Momordica charantia* varieties, Cyber titer 1:200, no. 2 varieties, Okinawa titer 1:200, no.3 varieties, Moddum titer 1:100, no. 4 varieties, Chinese bitter melon titer 1:100, no.5 *Psophocarpus tetragonolobus* varieties Green-winged bean titer 1:50, no.6 varieties Purple-winged bean titer 1:40, and no.7 *Ulex europaeus* titer 1:2 in NSS to dilute the antisera. Inactivated saliva (50 µL) was placed into 7 test tubes (10 x 75 mm) labelled 1, 2, 3, 4, 5, 6, and 7. Anti-H lectins (50 µL) were added to the corresponding test tubes, which were mixed and incubated at RT for 10 minutes to allow neutralization. Thereafter, 50 µL of 3% O cells are added to every test tube, incubated at RT for 30 minutes, centrifuged, and the results are interpreted.

Specificity testing method[3,5,6]

We selected the seed extraction that contains anti-H lectins from *Momordica charantia* Cyber, Moddam, Okinawa, Chinese bitter melon, and anti-H lectins from *Psophocarpus tetragonolobus*, Green and Purple-winged bean were compared to *Ulex europaeus* for specific testing. The optimal dilution of the seed-extracted anti-H

lectin was determined by performing a 2-fold dilution and testing with the corresponding standard RBCs: A, B, O, AB, Ahm, Bhm, and Ohm. The selection for the dilutions of the agglutination results was 1+ (score 5)[6] with A, 2+ to 3+ (score 8 to 10)[6] with B, 4+ (score 12)[6] with O, and negative with Ahm, Bhm, and Ohm RBCs. The optimal dilution of all six anti-H lectins was titer 1:16, prepared in 4% Bovine serum albumin, pH 7.2. The specificity was divided into two parts. Part 1 compared seven anti-H lectins: *Momordica charantia* Cyber, Moddam, Okinawa, Chinese bitter melon, and anti-H lectins *Psophocarpus tetragonolobus* Green and purple-winged bean, with 100 samples of A, B, O, and AB RBCs compared to *Ulex europaeus* with 50 samples of A, B, O, and AB RBCs. Part 2 compared three anti-H lectins: *Momordica charantia* Cyber, Okinawa, with 500 samples of A, B, O, and AB RBCs, and *Ulex europaeus* with 20 samples of A, B, O, and AB RBCs.

Stability testing method[5]

The chosen anti-H lectins *Momordica charantia* from Cyber and Okinawa titer 1:16, were stored at 4°C, RT, and 37°C; and tested for potency every three months for two years. Interval potency testing of antibodies stored at different temperatures was examined by titrating anti-H lectins against corresponding A, B, O, and AB RBCs using the standard tube test method.

Adsorption and heat elution method[7,8]

The adsorption and heat elution method confirmed that the seed extractions are truly anti-H lectins. We selected Ohm and A_3B RBCs for adsorption and heat elution. Anti-H lectins used were *Momordica charantia* Cyber, Moddam, Okinawa titer 1:16, and Chinese bitter melon titer 1:30, and anti-H lectins *Psophocarpus tetragonolobus* Green and Purple-winged bean titer 1:2 compared to *Ulex europaeus* concentrate.

Testing with ABO subgroup, para-Bombay, and cord blood

Anti-H lectins from *Momordica charantia* Cyber and Okinawa were tested against ABO subgroups A_2, A_3, B_3, A_2B, A_1B_3, A_3B, para-Bombay RBCs, and cord blood.

Testing results of anti-H lectin in patients

We gave anti-H lectins from *Momordica charantia* Cyber and Okinawa to the Transfusion Medicine Unit, King Chulalongkorn Memorial Hospital, Thai Red Cross Society, for testing the reactions and compared the results with anti-H murine monoclonal clone 5A9. They tested with 28 group A, 39 group B, 30 group O, and 5 group AB patients.

Trial production of anti-H lectin, five production lots.

Trial production of Anti-H lectin was carried out for five lots (68010, 68011, 68012, 68013, and 68014), followed by titration testing over 3 months. The total scores for each lot were then compared to determine whether there were any differences in the potency of O, B, AB, and A RBCs. The HAI tested 13 samples.

Results

This study found anti-H in four varieties of bitter gourd (*Momordica charantia*): Cyber, Moddam, Okinawa, Chinese bitter melon, and two varieties of *Psophocarpus tetragonolobus*: Green and purple-winged bean. The selected anti-H antibody, *Momordica charantia,* was titrated against A, B, O, AB, Ahm, Bhm, and Ohm RBCs. The extracts of every anti-H *Momordica charantia* showed hemolysis. The suitable dilution of anti-H *Momordica charantia* was Cyber 1:64, Moddam 1:16, Okinawa, and Chinese bitter melon 1:32, as shown in Table 1.

The HAI test of six seed extracts with those of *Ulex europaeus*. The results using 206 saliva samples showed 146 secretors and 60 non-secretors, with results identical to those of *Ulex europaeus* (100%). *Momordica charantia*: Moddum had 135 secretor samples, and 71 non-secretor samples showed different results, whereas *Ulex europaeus* had 11 samples (5.33%). *Momordica charantia*, Chinese bitter melon, had 136 secretor samples and 70 non-secretor samples and showed different results, as with *Ulex europaeus* (10 samples, 4.85%). *Psophocarpus tetragonolobus*, Green and Purple-winged bean, had 116 secretor samples, and 90 non-secretor samples showed different results, as *Ulex europaeus* 30 samples (14.56%). The results are shown in Table 2.

Table 1 Titration results of anti-H lectin from *Momordica charantia* seeds

Momordica charantia varieties	RBCs	Anti-H lectin titration									
		N	2	4	8	16	32	64	128	256	512
Cyber (1:64)	A	H	12(H)	12	12	10	7	5	0	0	0
	B	H	12(H)	12	12	11	9	6	0	0	0
	O	H	H	12	12	12	12	12	10	6	5
	AB	H	12(H)	12	12	12	3	8	5	0	0
	Ahm	H	5(H)	5	3	0	0	0	0	0	0
	Bhm	H	H	7	5	5	3	0	0	0	0
	Ohm	H	5(H)	8	5	5	0	0	0	0	0
Moddum (1:16)	A	H	12 (H)	11	10	8	4	0	0	0	0
	B	H	12 (H)	12	12	10	8	5	0	0	0
	O	H	12 (H)	12	12	12	12	10	6	4	0
	AB	H	12 (H)	10	8	5	3	0	0	0	0
	Ahm	H	2 (H)	5	0	0	0	0	0	0	0
	Bhm	H	5	5	0	0	0	0	0	0	0
	Ohm	H	5	5	0	0	0	0	0	0	0
Okinawa (1:16)	A	H	H	H	11	11	7	7	0	0	0
	B	H	H	H	12 (H)	12	11	8	5	0	0
	O	H	H	H	12	12	12	12	11	5	0
	AB	H	H	H	10	10	5	3	0	0	0
	Ahm	H	H	H	0	0	0	0	0	0	0
	Bhm	H	H	H	H	0	0	0	0	0	0
	Ohm	H	H	H	H	0	0	0	0	0	0
Chinese bitter melon (1:16)	A	H	H	H	H	H	5	7	0	0	0
	B	H	H	H	H	H	9	5	5	0	0
	O	H	H	H	H	H	12	11	8	4	0
	AB	H	H	H	H	H	5	3	0	0	0
	Ahm	H	H	H	H	H	0	0	0	0	0
	Bhm	H	H	H	H	H	0	0	0	0	0
	Ohm	H	H	H	H	H	0	0	0	0	0

Grading = Score: 4+ = 12, 3+ = 10, 2+ = 8, 1+ = 5, 1+w = 4, W = 2, 0 = 0

Table 2 Results of hemagglutination inhibition tests

Anti-H lectin	Secretor	Non-secretor
Momordica charantia Cyber	146	60
Momordica charantia Okinawa	146	60
Momordica charantia Moddum	135	71
Momordica charantia Chinese bitter melon	136	70
Psophocarpus tetragonolobus Green-winged bean	116	90
Psophocarpus tetragonolobus Purple-winged bean	116	90
Ulex europaeus	146	60

Specificity results were divided into 2 parts, part 1 compared anti-H lectins *Momordica charantia* Cyber, Moddam, Okinawa, Chinese bitter melon, *Psophocarpus tetragonolobus* Green and Purple-winged bean, and anti-H *Ulex europaeus*. The sum and average scores results are anti-H *Momordica charantia* Cyber with RBCs A (950, 9.50), B (1127, 11.27), O (1200, 12.00), AB (847, 8.47), anti-H *Momordica charantia* Okinawa with RBCs A (953, 9.53), B (1118, 11.18), O (1176, 11.76), AB (846, 8.46), anti-H *Momordica charantia* Moddum with RBCs A (987, 9.87), B (1139, 11.39), O (1200, 12.00), AB (865, 8.65), anti-H *Momordica charantia* Chinese bitter melon with RBCs A (505, 5.05), B (931, 9.31), O (960, 9.60), AB (676, 6.76), anti-H *Psophocarpus tetragonolobus* Green-winged bean with RBCs A (788, 7.88), B (947, 9.47), O (1155, 11.55), AB (684, 6.84), anti-H *Psophocarpus tetragonolobus* Purple-winged bean with RBCs A (969, 9.69), B (1085, 10.85), O (1188, 11.88), AB (846, 8.46) and anti-H *Ulex europaeus* with RBCs A (207, 4.14), B (265, 5.30), O (600, 12.00), AB (156, 3.12) shown in Table 3. Part 2 compared three anti-H lectins, *Momordica charantia* Cyber, Okinawa and *Ulex europaeus*. Sum and average scores results are anti-H *Momordica charantia* Cyber with RBCs A (3355, 6.71), B (5319, 10.64), O (6000,12.00), AB (3040, 6.08), anti-H *Momordica charantia* Okinawa with RBCs A (3326, 6.65), B (5308, 10.62), O (6000, 12.00), AB (3036, 6.07) and anti-H *Ulex europaeus* with RBCs A (92, 4.60), B (108, 5.40), O (240, 12.00) and AB (66, 3.3) shown in Table 3.

Stability results of anti-H *Momordica charantia* Cyber are shown in Figure 1. The sum scores at 4°C 3rd, 6th, 9th, 12th, 15th, 18th, 21st and 24th month were 91, 70, 64, 64, 72, 66, 56 and 52 at RT were 72, 55, 54, 56, 35, 33 34 and 36 at 37°C were 71, 50, 26, 18, 0, 0, 0 and 0. Stability results of anti-H *Momordica charantia* Okinawa are shown in Figure 2. The sum scores at 4°C 3rd, 6th, 9th, 12th, 15th, 18th, 21st and 24th month were 90, 70, 70, 65, 66, 48, 42 and 38 at RT were 83, 66, 57, 48, 44, 37, 37 and 28 at 37°C were 88, 50, 31, 31, 0, 0, 0 and 0.

Adsorption and heat elution results of Ohm cells score 3 all every anti-H lectins, and A_3B cells score 10 were anti-H *Momordica charantia* Cyber, Okinawa, Chinese bitter melon, and *Ulex europaeus*, and score 12 were *Momordica charantia* Moddum, *Psophocarpus tetragonolobus* green and purple- winged bean. The last all supernatant control was negative, and all elution is shown in Table 4. Testing anti-H *Momordica charantia* Cyber and Okinawa with ABO subgroup, para-Bombay RBCs, and cord blood is shown in Table 5. A_3, B_3 cells average score 12.0. A_2, A_2B, A_3B, A_1B_3 average score 10.0 to 11.6. Ahm, Bhm, and Ohm were negative, and cord blood was 12.0.

The testing results for anti-H lectins in patients from the Transfusion Medicine Unit, King Chulalongkorn

Table 3 Specificity results of anti-H lectin

RBCs	Scores	Part 1							Part 2		
		Momordica charantia				*Psophocarpus tetragonolobus*		*Ulex europaeus*	*Momordica charantia*		*Ulex europaeus*
		Cyber	Okinawa	Moddum	Chinese bitter melon	Green-winged bean	Purple-winged bean		Cyber	Okinawa	
A	Sum	950	953	987	505	788	969	207	3,355	3,326	92
	Averages	9.50	9.53	9.87	5.05	7.88	9.69	4.14	6.71	6.65	4.60
B	Sum	1,127	1,118	1,139	931	947	1,085	265	5,319	5,308	108
	Averages	11.27	11.18	11.39	9.31	9.47	10.85	5.30	10.64	10.62	5.40
O	Sum	1,200	1,176	1,200	960	1,155	1,188	600	6,000	6,000	240
	Averages	12.00	11.76	12.00	9.60	11.55	11.88	12.00	12.00	12.00	12.00
AB	Sum	847	846	865	676	684	846	156	3,040	3,036	66
	Averages	8.47	8.46	8.65	6.76	6.84	8.46	3.12	6.08	6.07	3.3

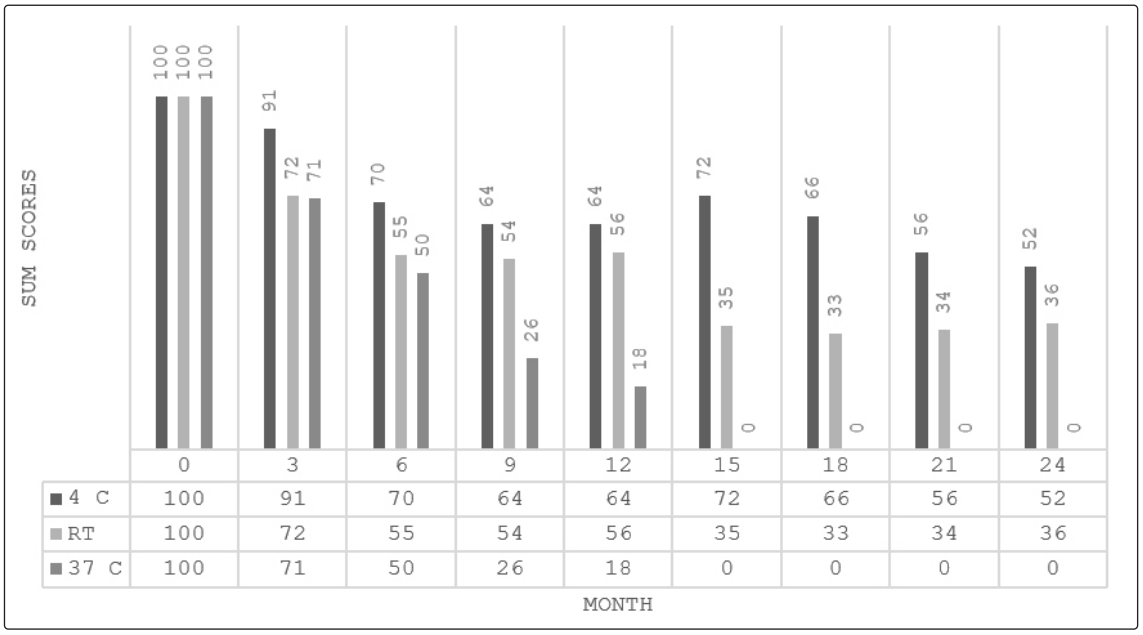

Figure 1 Stability at different temperatures of anti-H lectin *Momordica charantia*: Cyber

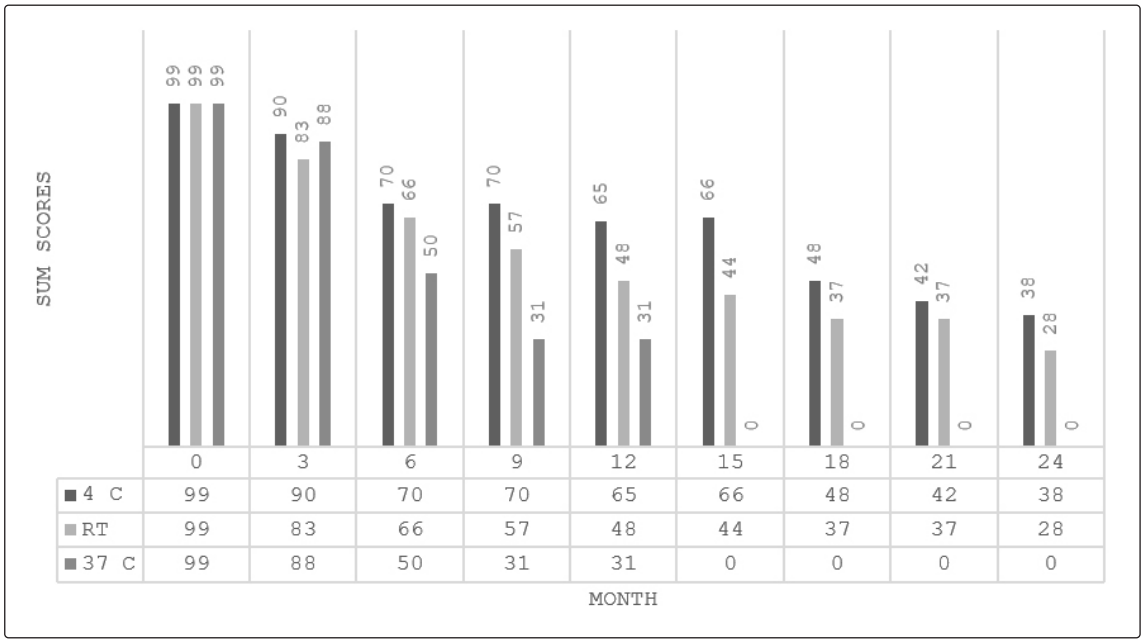

Figure 2 Stability at different temperatures of anti-H lectin *Momordica charantia*: Okinawa

Table 4 Results of anti-H adsorption and heat elution

Eluate from	Anti-H absorption						
	Momordica charantia				*Psophocarpus tetragonolobus*		*Ulex europaeus*
	Cyber	Okinawa	Moddum	Chinese bitter melon	Green-winged bean	Purple-winged bean	
Ohm RBCs	3	3	3	3	3	3	3
A_3B RBCs	10	10	12	10	12	12	10
Control	0	0	0	0	0	0	0

Table 5 Testing anti-H lectin from *Momordica charantia* with ABO subgroup and para-Bombay RBCs

RBCs	Number	Anti-H *Momordica charantia* average scores	
		Cyber	Okinawa
A_3	10	12.0	12.0
B_3	12	12.0	12.0
A_2	5	11.6	11.6
A_2B	5	11.2	11.0
A_3B	2	10.0	11.0
A_1B_3	2	11.5	11.5
Ahm	3	0	0
Bhm	2	0	0
Ohm	2	0	0
Cord	1	12.0	12.0

Table 6 Testing results of anti-H lectin in patients

Patients	N	Anti-H Murine monoclonal Clone 5A9		Anti-H lectin *Momordica charantia* Cyber		Anti-H lectin *Momordica charantia* Okinawa	
		Sum scores	Average scores	Sum scores	Average scores	Sum scores	Average scores
Group A	28	220	7.86	324	11.57	320	11.43
Group B	39	349	8.95	466	11.95	466	11.95
Group O	30	360	12.00	360	12.00	360	12.00
Group AB	5	23	4.60	56	11.20	50	10.00

Memorial Hospital, Thai Red Cross Society are shown in Table 6. The sum and average scores results are anti-H murine monoclonal with patients group A (220, 7.86), B (349, 8.95), O (360, 12.00), AB (23, 4.60), anti-H *Momordica charantia* Cyber with patients group A (324, 11.57), B (466, 11.95), O (360, 12.00), AB (56, 11.20), anti-H *Momordica charantia* Okinawa with patients group A (320, 11.43), B (466, 11.95), O (360, 12.00), AB (50, 10.00) show in Table 6.

The titration scores of the five lots of anti-H lectin (lot 68010, 68011, 68012, 68013, and 68014) are shown in Table 7. The total titration scores of anti-H lectins were as follows: lot 68010: reactivity with cells O > B, AB > A = 87 > 43, 49 > 37; lot 68011: reactivity with cells O > B, AB > A = 93 > 55, 50 > 45; lot 68012: reactivity with cells O > B, AB > A = 84 > 47, 46 > 42; lot 68013: reactivity with cells O > B, AB > A = 83 > 40, 39 > 33; lot 68014: reactivity with cells O > B, AB > A = 85 > 42, 35 > 27. The HAI of anti-H lectin (lot 68010, 68011, 68012, 68013, and 68014) of 13 samples are 11 samples are secretors, and two samples are non-secretors.

The comparison, grading, and score titration shown under Table 1 are from the AABB technical manual, 15th edition, Table 1-8-1. Interpretation of agglutination in method 1-8: reading and grading tube agglutination.[6]

Discussion

Anti-H lectin *Ulex europaeus* is a reagent for H antigen typing on RBCs and H substance inhibition in saliva. It is a necessary reagent for the blood bank laboratories. In the past, the Antiserum and Standard Cells Production Section produced this reagent for hospital blood bank laboratories in Thailand by importing *Ulex europaeus* seeds from Europe, but we can no longer obtain them. *Ulex europaeus* is an European native plant that cannot be grown in tropical areas.

Table 7 Trial production of anti-H lectin

Anti-H lectin	RBCs	Titration sum scores			Total Sum scores
		Month 1	Month 2	Month 3	
Lot. 68010	A	12	10	15	37
Exp. 10/07/26	B	13	15	15	43
	O	37	26	24	87
	AB	15	20	14	49
	Ohm	0	0	0	0
Lot. 68011	A	13	16	16	45
Exp. 10/07/26	B	16	22	17	55
	O	35	30	28	93
	AB	14	18	18	50
	Ohm	0	0	0	0
Lot. 68012	A	13	15	14	42
Exp. 10/07/26	B	16	16	15	47
	O	35	23	26	84
	AB	13	16	17	46
	Ohm	0	0	0	0
Lot. 68013	A	8	15	10	33
Exp. 10/07/26	B	8	16	16	40
	O	30	27	26	83
	AB	10	13	16	39
	Ohm	0	0	0	0
Lot. 68014	A	7	14	6	27
Exp. 10/07/26	B	13	15	14	42
	O	34	27	24	85
	AB	12	11	12	35
	Ohm	0	0	0	0

We developed a murine monoclonal anti-H to replace *Ulex europaeus*. Still, it cannot inhibit H substances in saliva.[2] This is because H substances are divided into two types of oligosaccharide chain: type 1 and type 2. Saliva and other body fluids are both H type 1 and H type 2, but RBC membranes synthesize only H type 2.[9] Anti-H murine monoclonal specifically H type 2 on RBCs cannot inhibit H type 1 in saliva, so HAI results are incorrect.

This study aims to identify anti-H lectins in seeds from local plants that can detect H substances in saliva corresponding to anti-H *Ulex europaeus*, the gold standard used in blood bank laboratories. The study showed that six local plant seeds contain anti-H lectins, including four varieties of *Momordica charantia*: Cyber, Moddam, Okinawa, and Chinese bitter melon, and the varieties of *Psophocarpus tetragonolobus*: Green and Purple-winged bean. Table 1 shows anti-H lectins from Cyber, Moddam, Okinawa, and Chinese bitter melon which the results titration with A, B, O, AB, Ahm, Bhm and Ohm RBCs are consistent with the theory of highest amount of H antigens group O[13], followed by group B, AB, and A, respectively, and shows negative results with Ahm, Bhm and Ohm RBCs. Hemolysis was observed in the early dilutions, and the cause remains unknown. The HAI test with H substance

in saliva that anti-H lectins from *Momordica charantia* Cyber and Okinawa showed the same results as anti-H lectin *Ulex europaeus* in all tested saliva samples. A total of 146 individuals were secretors and 60 were non-secretors, consistent with the findings reported by Kong SP and colleagues. However, some samples of anti-H *Momordica charantia* (Moddam, Chinese bitter melon) and *Psophocarpus tetragonolobus* (Green and Purple winged bean) showed discrepant results with anti-H lectins from *Ulex europaeus*. In 1953, Morgan and Watkins found that L-fucose inhibited some seed anti-H reagents, indicating that H-specificity was an important determinant.[10,11] Lectins in *Ulex europaeus* are classified into two types: Ulex I and Ulex II. L-fucose inhibited Ulex I but did not inhibit Ulex II. Sugar with an N-acetyglucosaminyl residue and di-N-acetylchitobiose inhibited Ulex II so lectins that are similar to Ulex I are inhibited by L-fucose, e.g., *Psophocarpus tetragonolobus* and Ulex II which are inhibited by N-acetylglucosamine derivatives, lectins that are similar to Ulex II e.g. *Cystisus sessilifolius* and *Laburnum alpinum*.[1,11] Ulex II does not specifically bind to the H antigen but specificity for the Type 2 precursor chain (Galβ1-4GlcNAc), which is a basic structure found in the ABO blood group system that is commonly found on RBCs surface of A_1 and B blood groups. Ulex II binds to the unconverted precursor structures, not to A or B antigens themselves, so it cannot be used for identifying Bombay or para-Bombay phenotypes, since it does not recognize the H antigen.[1,12] *Psophocarpus tetragonolobus* and *Momordica charantia* Moddam, Chinese bitter melon lectins are not suitable to produce anti-H reagents for saliva testing. Therefore, *Momordica charantia* Cyber and Okinawa lectins appear more ideal to produce anti-H lectin reagents, as HAI results are the same as those with *Ulex europaeus*.

The specificity resulting from anti-H is divided into two parts because the selected anti-H lectin candidate for reagent production will be tested on a larger number of blood samples. The observed specificity of every anti-H lectin corresponded with the theoretical distribution of H antigen among different ABO blood groups. Anti-H lectins from *Momordica charantia*: Cyber average sum scores were RBCs: A 6.71, B 10.64, O 12.00, and AB 6.08. Anti-H lectins from *Momordica charantia*: Okinawa average sum scores were RBCs: A 6.65, B 10.62, O 12.00, and AB 6.07. These two anti-H lectins showed comparable and highly similar reactivity toward the ABO H antigens, indicating equivalent specificity. However, this differs from anti-H lectins, such as *Ulex europaeus*, which show stronger reactivity with group O than with other blood groups. The results of Phatak, et al. (2014) showed that *Momordica charantia* anti-H lectins were strongly agglutinated to all ABO blood groups. In contrast, *Ulex europaeus* anti-H lectins agglutinated strongly to the O blood group more than to other blood groups.[13]

The stability of anti-H lectins from *Momordica charantia* Cyber and Okinawa was performed at 4 ^{O}C, RT, and 37 ^{O}C at three-month intervals over a two-year period. The stability study showed that both types of anti-H lectins demonstrated comparable stability. The percentage decreased by approximately 10% during the first three months, remained decreased at approximately 30% in the sixth month, and decreased by approximately 40%, leaving 60% of the original potency in the 18th month. There was a slight difference in potency between anti-H lectins from *Momordica charantia* Cyber and Okinawa by the 18th month; the potency of anti-H lectins from Cyber decreased to 60% while anti-H lectins from Okinawa decreased to 40% so anti-H lectins from Cyber were more stable than anti-H lectins from Okinawa. Consequently, anti-H lectin Cyber is considered more appropriate for reagent production, as its shelf life has been defined at 18 months.

The results of anti-H adsorption and heat elution using Ohm and A_3B RBCs demonstrate that the lectins extracted from *Momordica charantia* and *Psophocarpus tetragonolobus* are truly anti-H. Ohm RBCs express fewer H antigens than A_3B cells, and therefore adsorb fewer anti-H lectins, resulting in a less potent eluate

and anti-H from *Momordica charantia* from Cyber and Okinawa tested with ABO subgroup and Ahm, Bhm, Ohm RBCs and cord blood. A_3, B_3, and cord blood exhibit the highest strength. The reactivity of A_2, A_2B, A_3B, and A_1B_3 RBCs is slightly less intense. Ahm, Bhm, and Ohm RBCs were negative. This finding is in accordance with the theory, since subgroup RBCs retain a substantial amount of H antigens, which have not been fully converted into A or B antigens.[2,13] For Ahm, Bhm, and Ohm RBCs, which have the lowest amount of H antigens on the RBC surface, the reaction yielded a negative result.[2] Cord blood naturally expresses more H antigens than adult RBCs, especially in the group and subgroup of A and B. This is because A and B transferase enzymes are undeveloped, so less H gets converted into A or B antigens in neonates.[14] The testing results of the anti-H lectins *Momordica charantia* Cyber and Okinawa in patients at King Chulalongkorn Memorial Hospital are comparable to those of Phatak et al. (2014). All ABO blood groups showed equal average scores.[13] The titration scores for all five lots of anti-H lectins were consistent with theoretical expectations. The O cells showed the highest reactivity, followed by B and AB cells, while A cells showed the lowest reactivity. The para-Bombay cells gave negative results in all lots. The HAI results were consistent across all lots.

In this study, anti-H was extracted from *Momordica charantia* using the most straightforward approach, saline extraction. Previous research relied on purification procedures that involved more elaborate, time-consuming steps.[13,15] Our goal was to develop a ready-to-use anti-H lectin reagent for convenient use in blood bank laboratories. The selection of *Momordica charantia* seeds for production is also of great importance. Initially, we used seeds sourced from local fresh markets, where the fruits were intended for culinary use, the same method as Phatak et al. (2014).[13] Still, we were unsuccessful because the extract induced complete hemolysis in all RBCs and could not be diluted. The failure was likely attributed to the use of seeds from immature fruits obtained at a local fresh market, which contained insufficient levels of anti-H lectins, resulting in an extract that could not be effectively diluted. Subsequently, we switched to using cultivation-grade seeds purchased from agricultural supply stores, sourced from fully mature fruits. This resolved the problem of RBCs hemolysis. Moreover, the extract could be diluted and titrated to high levels of anti-H lectins, as shown in Table 1. The maturation of *Momordica charantia* seeds does not occur uniformly. If seeds are harvested too early or too late, they may be either immature or overly mature, resulting in reduced seed quality and yield. This is because the seeds may not have fully matured or may have already begun to deteriorate in quality. Harvesting seeds at the appropriate stage ensures good seed quality, as the timing of harvest is a critical determinant of seed quality. Seeds reach their highest quality during physiological maturity, which is when they have accumulated the maximum dry weight.[16] The decision to use *Momordica charantia* seeds with cultivation grade is appropriate because seed companies have already selected and ensured that the seeds they offer for sale are of the highest quality. Moreover, the seeds contain a higher level of anti-H lectin than those purchased from local fresh markets. In conclusion, *Momordica charantia* from Cyber is the most suitable seed for producing anti-H lectin reagents for use in blood bank laboratories due to its high titration and good stability.

Acknowledgement

We wish to thank Mrs. Sarika Makechay, Assistant Head of Antiserum and Standard Cells Production Section, and Mrs. Jintana Tubrod for their consultation on this research.

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