BULLETIN OF TAIPEI MEDICAL COLLEGE, VOL. 15, MAY 1986
*P. 119-126*

# Hemagglutination Activities of the Seed Extracts of *Litchi chinensis* and *Euphoria Longana*

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**SUMMARY**

*The crude extracts of the seeds of litchi and longan were prepared by extraction with 5% acetic acid solution, and the extracts were neutralized and filtered. Fractions of litchi and longan crude extracts were obtained by fractional ammonium sulfate precipitation. Hemagglutination titers of the crude extracts and ammonium sulfate precipitated fractions toward erythrocytes of human, rat, and sheep were determined. Litchi crude extract was applied to a Sephadex G-50 column (2 x 10cm) and separated into Frs. I, II, III and IV by stepwise washing with 0.05 M Na phosphate pH 7.0, 0.5 N acetic acid, 1.0 N acetic acid, and 0.1 N NaOH solutions. Longan crude extract was separated into Frs. I and II by Sephadex G-50 column (2.2 x 16cm) chromatography using 0.01 M Na phosphate as eluant. Hemagglutination titers of the fractions obtained by Sephadex G-50 column chromatography were also determined. Hemagglutination titers of the crude extracts and their fractions indicated that the seeds of litchi or longan had different kinds of hemagglutinin. The interactions of Sephadex G-50 with the components of litchi and longan seed extracts were described.*

*Key words:* ***Litchi chinensis, Euphoria Longana,*** *Seed extract, Hemagglutination, Sephadex G-50.*

Lectins of plant origin, owing to their biological activities, have been widely used as research tools in the fields such as mechanism of mitogenic action, cancer therapy, and cell membrane structure[1]. Surveys on the lectin contents of plants for providing new lectin sources had been undertaken in many areas of the world[2,3]. More than four thousand seed plants had been identified on the island of Formosa and its adjacent islands.[4]. Recently, we had just begun a survey work for lectin contents of native seed plants in Taiwan area. The lectin contents of 111 species of native plants in Taiwan had been studied by Wang and Liu in 1975[5]. According to their report, saline seed extracts of litchi (*Litchi chinensis*) and longan (*Euphoria*

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Received for Publication: January 20, 1986.

*Longana*) could agglutinate the erythrocytes of human. In this work, we had extracted the seeds of litchi and longan with 5% acetic acid solution. Hemagglutination titers of the seed extracts and their fractions obtained by ammonium sulfate precipitation or Sephadex G-50 column chromatography were determined.

## MATERIALS AND METHODS

The fruits of litchi and longan were obtained from the fruit market in Taipei area. The fruit seeds were dried under sunshine and ground in a mortar before use. Two purified lectins used in this work for the comparison of hemagglutination activity were concanavalin A (*Canavalia ensiformis* lectin) and *Canavalia lineata* lectin. Concanavalin A purified by the method of Olson and Liener[6] was purchased from Sigma company. *Canavalia lineata* lectin was purified by binding the lectin in the bean meal extract to a Sephadex G-50 column and the bound lectin was eluted with 0.05 M maltose solution[7]. Sephadex G-50 was obtained from Pharmacia Fine Chemicals.

Preparation of the crude extracts: Fifty gm ground seed of litchi or longan was extracted with 500 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 again to remove the precipitate. For hemagglutination titer determination, the crude extract was dialyzed against distilled water and lyophilized.

Fractionation of the crude extracts by ammonium sulfate precipitation: Solid ammonium sulfate powder (E. Merck) was added to the crude extract of litchi or longan to desired concentration at room temperature. The precipitate formed was collected by centrifugation (10,000 x g) in a Hitachi high speed centrifuge, and dissolved in distilled water. The solution was dialyzed against distilled water and lyophilized.

Determination of hemagglutination titer: Red blood cells of human, albino rat, or sheep were collected in and washed with phosphate buffered saline pH 7.2 (PBS) just before use. Hemagglutination was performed in a U-shape well microtiter plate (Cook engineering company). 0.1 ml two-fold dilutions of a sample under test in PBS and 0.1 ml 1% red blood cell suspension in PBS were mixed and incubated at 37°C for one hour. The minimal concentration of the sample (mg/ml) which could induce hemagglutination (end point) was judged from the settling patterns of red blood cells. Hemagglutination titer of the sample was expressed in the reciprocal of this concentration. Two-fold difference in hemagglutination titer of two samples was not considered to be significantly different, since it was within the experimental error of judging the end point.

## RESULTS

**Hemagglutination titers of the crude extracts and ammonium sulfate precipitated fractions:**

The results of hemagglutination titer

determination were shown in Table 1. Concanavalin A and *Canavalia lineata* lectin, which were used as references for the comparison of hemagglutination activity, had similar or identical hemagglutination titers toward the erythrocytes of the same species of animal or same type of human origin. The red blood cells of sheep were most sensitive to these two lectins.

The hemagglutination titers of litchi crude extract toward rat and sheep erythrocytes were much lower than those of two reference lectins. The results also showed that litchi crude extract could agglutinate equally well with different types of human erythrocytes. Hemagglutination titers of 0 to 100% ammonium sulfate saturation precipitate were 8 times higher than that of the crude extract with respect to the erythrocytes of rat and human. This fact suggested that ammonium sulfate precipitation could remove some undesired substance and concentrate the component which had hemagglutination activity. In an attempt to get the better result, we had obtained two fractions from the crude extract by fractional ammonium sulfate precipitation (0 to 50% saturation, and 50 to 100% saturation). With respect to the erythrocytes of rat and sheep, hemagglutination titers

**Table 1. Hemagglutination Titers of Seed Extracts and Ammonium Sulfate Precipitated Fractions**

| Fraction | Hemagglutination Titer | | | | | |
|---|---|---|---|---|---|---|
| | Erythrocyte | | | | | |
| | Rat | Sheep | Human | | | |
| | | | O | A | B | AB |
| Concanavalin A | 512 | 2048 | 4 | 64 | 64 | 16 |
| *Canavalia lineata* lectin | 256 | 4096 | 4 | 64 | 64 | 16 |
| *Litchi chinensis* | | | | | | |
| Crude extract | 32 | 32 | 64 | 64 | 64 | 32 |
| % ammonium sulfate saturation ppt. | | | | | | |
| 0 to 100 | 256 | 64 | 512 | 512 | 256 | 256 |
| 0 to 50 | 128 | 32 | 256 | 256 | 256 | 256 |
| 50 to 100 | 256 | 64 | 1024 | 1024 | 512 | 512 |
| *Euphoria Longana* | | | | | | |
| Curde extract | 512 | 256 | <2 | 4 | 64 | 128 |
| % ammonium sulfate saturation ppt. | | | | | | |
| 0 to 100 | 512 | 256 | 64 | 64 | 128 | 512 |
| 0 to 50 | 512 | 256 | 64 | 32 | 128 | 256 |
| 50 to 100 | 512 | 128 | 32 | 32 | 256 | 256 |
| 0 to 20 | 1024 | 512 | 64 | 128 | 256 | 512 |
| 20 to 60 | 1024 | 512 | 64 | 128 | 256 | 512 |
| 60 to 100 | 512 | 512 | 32 | 64 | 128 | 256 |

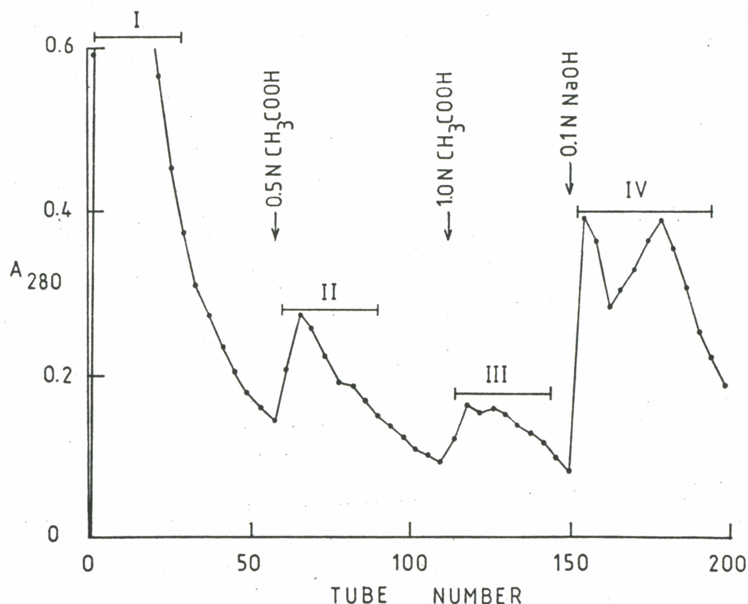


Fig. 1. Sephadex G-50 column (2 x 10cm) chromatography of litchi crude extract. 50 ml litchi crude extract was applied to the column and stepwise eluted with 0.05M Na phosphate pH 7.0, 0.5N acetic acid, 1.0N acetic acid, and 0.1N NaOH solutions. The flow rate was 15ml/tube/12min. Four fractions collected were indicated by the horizontal bars.

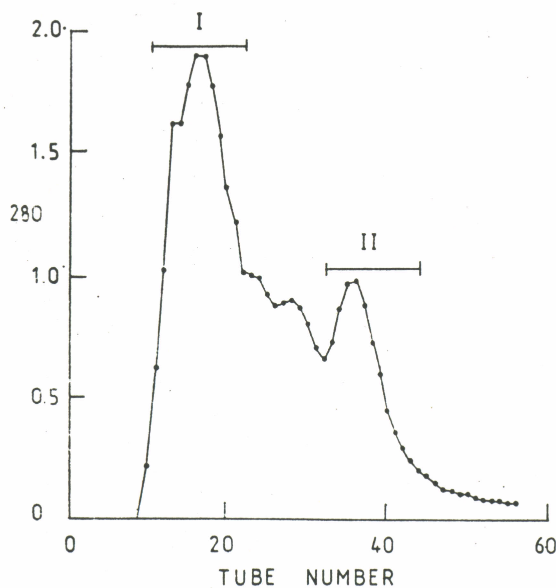


Fig. 2. Sephadex G-50 column (2.2 x 16cm) chromatography of longan crude extract. 16mg lyophilized longan crude extract in 2ml 0.01M Na phosphate buffer pH 7.0 was applied to the column and eluted with the same buffer at a flow rate of 4ml/tube/8min. Two fractions collected were indicated by the horizontal bars.

of these two fractions were not higher than that of 0 to 100% saturation precipitate. However, if we compared these two fractions with the crude extract, the 50 to 100% saturation precipitate had better purification effect. This fraction was 8-fold or 16-fold purified with respect to human erythrocytes as compared with the crude extract.

The hemagglutination titer of longan crude extract was 16 times higher than that of litchi crude extract with respect to rat erythrocytes, and 8 times higher with respect to sheep erythrocytes. All fractions obtained by ammonium sulfate precipitation had equal or slightly higher hemagglutination titers toward rat and sheep erythrocytes as compared with the crude extract. An inspection of hemagglutination titers of the crude extract and ammonium sulfate precipitated fractions toward different types of human erythrocytes, we found that AB-type erythrocyte was most sensitive, and B-type erythrocyte was slightly more sensitive than A-type and 0-type erythrocytes. Furthermore, the results also showed that ammonium sulfate precipitation could increase the hemagglutination titers toward human erythrocytes, especially with respect to O-type and A-type erythrocytes.

**Sephadex G-50 column chromatography of litchi crude extract:**

Fifty ml litchi crude extract was dialyzed against distilled water and 0.05 M Na phosphate buffer pH 7.0, then applied to a Sephadex G-50 column (2 x 10cm). The column was eluted with 0.05 M Na phosphate buffer pH 7.0 to wash out the components which were not retained by the Sephadex column. The components which were retained by the column were eluted stepwise with 0.5 N acetic acid, 1.0 N acetic acid, and 0.1 N NaOH solutions. The elution rate was 15 ml/tube/12 minutes. The elution profile was shown in Fig. 1 and four fractions were collected from the effluent. The fractions were dialyzed against distilled water and lyophilized. Hemagglutination titers of the fractions were shown in Table 2. The titers of Frs. I, II, and III were equal to or slightly different from that of the crude extract. The titer of Fr. IV increased 4 or 8 times with respect to rat or sheep erythrocytes as compared with the crude extract. However, the hemagglutination activity of this fraction toward human erythrocytes was obviously decreased.

**Sephadex G-50 column chromatography of longan crude extract:**

Sixteen mg lyophilized longan crude extract was dissolved in 2 ml 0.0l M Na phosphate buffer pH 7.0 and applied to a Sephadex G-50 column (2.2 x 16cm). The column was eluted with 0.01 M Na phosphate buffer pH 7.0 at a flow rate of 4 ml/tube/8 minutes. The elution profile was shown in Fig. 2 and two fractions were collected from the effluent. The fractions were dialyzed against distilled water and lyophilized. Hemagglutination titers of the fractions were shown in Table 2. With respect to rat and sheep erythrocytes, the titers of Frs. I and II were lower than that of the crude extract. Although both fractions showed stronger agglutination activity toward O-type and A-type human

**Table 2. Hemagglutination Titers of the Fractions Obtained by Sephadex G-50 Column Chromatography of Seed Extracts**

| Fraction | Hemagglutination Titers | | | | | |
|---|---|---|---|---|---|---|
| | Erythrocyte | | | | | |
| | Rat | Sheep | Human | | | |
| | | | O | A | B | AB |
| *Litchi chinensis* | | | | | | |
| Crude extract | 32 | 32 | 64 | 64 | 64 | 32 |
| Fr. I | 32 | 64 | 32 | 64 | 64 | 64 |
| Fr. II | 32 | | 64 | 16 | 16 | 8 |
| Fr. III | 64 | 32 | 128 | 128 | 32 | 32 |
| Fr. IV | 128 | 256 | <2 | 4 | 4 | 4 |
| *Euphoria Longana* | | | | | | |
| Crude extract | 512 | 256 | <2 | 4 | 64 | 128 |
| Fr. I | 128 | 32 | 8 | 8 | 2 | <2 |
| Fr. II | 64 | 32 | 16 | 32 | 32 | 8 |

erythrocytes, the activity of Fr. I toward B-type and AB-type erythrocytes was very weak, and the titer of Fr. II toward AB-type erythrocyte was 16 times lower than that of the crude extract.

## DISCUSSION

The method of extraction which was used in the purification of lectin from the bean meal of *Canavalia lineata*[7] was applied to the seeds of litchi and longan in this study, and the results were satisfactory. This extraction procedure is used in our survey work for lectin content in plants of Taiwan.

The hemagglutinin in litchi crude extract can be precipitated by ammonium sulfate, and ammonium sulfate precipitated fractions have higher hemagglutination titers than that of the crude extract. The effect of fractional precipitation by ammonium sulfate on the purification of litchi hemagglutinin is not clear-cut because of the responses of erythrocytes from different species are not concerted (Table 1). This fact may indicate that hemagglutination activity of litchi seed extract is not due to a single component, and different hemagglutinins have different specificities toward erythrocytes of different sources. This propose is supported by the results of hemagglutination activities of the four fractions obtained by Sephadex G-50 column chromatography (Table 2). All of the four fractions have hemagglutination activity, but the specificity toward erythrocytes of different sources is not identical. For example, Fr. IV has high hemagglutination activities toward rat and sheep erythrocytes, but its titers with respect to the erythrocytes of human origin are very low. Similar results have been obtained from longan crude extract and its fractions obtained by fractional ammonium sulfate precipitation or Sephadex G-50 column

chromatography (Tables 1 and 2). The seed extract of longan may also contain different hemagglutinins is a reasonable interpretation of the results.

As shown in Fig. 1, Frs. II, III, and IV are the components in litchi crude extract which are retained by the Sephadex G-50 column. After Fr. IV is eluted out of the column with 0.1 N NaOH solution, a colored band is still remained in the upper part of the column. Although Fr. I can be eluted out of the column with the phosphate buffer, the substance of this fraction can also interact with Sephadex G-50, since the elution volume of this fraction is far larger than the bed volume of the column (31 ml) plus sample volume (50 ml). Furthermore, we have found that the interaction between the components of litchi crude extract and Sephadex G-50 is not interfered in the presence of 0.1 M maltose. Therefore, this type of interaction is different from the interaction of Sephadex G-50 with concanavalin A[8] or *Canavalia lineata* lectin[7].

From the results of Sephadex G-50 column chromatography of longan seed extract (Fig. 2), substances of Frs. I and II can also interact with Sephadex G-50, since the elution volumes of these fractions are larger than the bed volume of the column (61 ml) plus sample volume (2 ml). The results of Sephadex G-50 column chromatography also reveal a strange phenomenon. Hemagglutination titers of Frs. I and II are much lower than that of longan crude extract with respect to rat and sheep erythrocytes (Table 2). The reason of this fact is not clear. Recently, we have attempted separating the components of longan seed extract by gel-filtration through a Bio-Gel P-150 column. This type of gel is selected because we expect that polyacrylamide will not interact with the components of longan seed extract, and better separative effect will be achieved. However, we find that most of the components interact strongly with the gel and are firmly retained by the column.

Based on the findings described above, the hydrophobic interaction may play an important role in the interaction of seed components of litchi and longan with the polymers.

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# 荔枝和龍眼種子抽取物之紅血球凝集活性

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五十公克荔枝或龍眼種子，以500毫升5%醋酸水溶液抽取其成分，抽取液經中和並透析後，冷凍乾燥而得到種子抽取物。荔枝種子抽取物對老鼠和羊的紅血球，都會引起凝集作用；對不同血型之人類紅血球，亦有凝集作用而且活性相同。龍眼種子抽取物對老鼠和羊的紅血球，有很好的凝集作用，其凝集活性比荔枝種子抽取物高8至16倍。但對不同血型之人類紅血球，有不同的凝集活性。對O型和A型紅血球之凝集活性較低，但對AB型及B型之紅血球則凝集活性較高。荔枝種子抽取物之飽和硫酸銨（0至100%）沉澱物，對各種紅血球都有較高的凝集活性。然而，0至50%飽和沉澱物和50至100%飽和沉澱物之紅血球凝集活性，並沒有顯著的差異。龍眼的種子抽取物，由飽和程度不同之硫酸銨溶液所得之各種沉澱物，對老鼠和羊的紅血球之凝集活性，約略和種子抽取物相同。但硫酸銨沉澱作用，可提高對人類紅血球的凝集活性，尤其是對於O型和A型的紅血球。利用Sephadex G-50柱層析法，可將荔枝種子抽取物之成分分為四個部份。這四個部份對各種紅血球，都具有不同程度的凝集作用。龍眼種子抽取物亦可以利用Sephadex G-50柱層析法，分為二個部份。此二部份對各種紅血球也具有凝集作用。但其活性都比種子抽取物低，其原因不詳。綜合以上實驗的結果，我們推測荔枝和龍眼的種子中，具有紅血球凝集作用的成分，可能不止一種。因為各種不同硫酸銨飽和程度所得之沉澱物，以及由Sephadex G-50柱層析法所得之各部份，都具有紅血球凝集的活性。此外，我們發現荔枝和龍眼種子的成分，可以和Sephadex G-50發生交互作用。荔枝種子成分之作用力較強，因此可應用於此類種子成分的分離。

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民國七十五年一月二十日受理