## CHAPTER IV

## DISCUSSION

As mentioned above, Niang beans are relished as a food or as a flavouring for food by local inhabitants. However, their chemical components and nutritive values have never been assessed. The result in the present studies showed that Niang beans contained low protein, low fat but high carbohydrate values. These data were very much different from those of pulses and nuts, but very similar to the chest nut and the water chest nut. Our results were in accordance with those of Meevasana (1966) and Sein Gwan and Chit Maung (1968). Niang beans also contained some vitamins (vitamins  $B_1$ ,  $B_2$ , C and folic acid) and minerals (calcium, phosphorus and iron).

Sein Gwan and Chit Maung (1966) reported that the beans contained essential amino acids, i.e., tryptophan, lysine, arginine, threonine, valine, leucine, and 6 non-essential amino acids. Later Suanpan (1973) was able to detect virtually all of essential amino acids and 9 non-essential amino acid from Niang bean. The result in the present study showed that Niang beans contained 18 amino acids including all 8 essential amino acids. All these data revealed that Niang beans provide a high nutritive values for human food.

Djenkolic acid could be extracted from the beans by homogenizing in the water or in 70% ethanol solution. The results showed that about 1.7-10.8% of djenkolic acid in the beans could be extracted. This figure was higher than the water extraction performed by West et al. (1973).

It has been shown that djenkolic acid occurs in a free state in Niang beans by Van Veen and Latusan (1949) and West et al. (1973). Our results also showed that about 93% of djenkolic acid was in the free state and only 7% was found to be bound to the protein.

West et al. (1973) managed to reduce the djenkolic acid content of the beans to a half value by boiling the beans in the sodium bicarbonate solution (5%) for 10 minutes. Our results also revealed that boiling Niang beans in water and 5% HCl for 10 minutes could reduce the djenkolic acid content to 30-32%. If the boiling times were prolonged to 20 minutes in 5% NaHCO<sub>3</sub>, the djenkolic acid content could be reduced further to 13%. These results were in accordance with the data presented by West et al. (1973).

As djenkolic acid occurs as the free acid, it is therefore, possible to remove a considerable amount of the djenkolic acid content of the djenkol beans by boiling in these solvents. As mentioned above, it was possible to reduce the djenkolic acid content of beans to 13% by boiling the cotyledon of one beans for 20 minutes in 5% sodium bicarbonate.

However, the usefulness of these treatments depend on the palatability of the beans to the people who like to consume such beans.

It has been known among the native that Niang beans may sometimes cause haematuria and anuria in some persons who were susceptible to this kind of beans. The symptoms appeared in 2-12 hours and were associated with the appearance of djenkolic crystals in the urine. Blockage of the renal tubules and ureters stops the production of urine. Vomiting diarrhea and fever may occur. Post-mortem examinations showed only minor changes in the kidneys. The kidneys may be edematous and cortical necrosis has been seen in child.

Only a few inconclusive studies of toxicity of Niang beans have been done. Van Veen and Hyman (1933) fed the beans to monkeys, but aside from crystalluria, no changes were observed. Mreyen (1941) fed beans to one monkey and found no definite findings. These results were very similar to the present studies. Only decreased urine cutput was observed in 5 monkeys fed with the extraction of Niang beans who otherwise proved to be normal. On the other hand, one of the two adult rhesus monkeys fed with 20% Niang for 1-2 weeks showed abundant rosettes of needle shaped crystals resembling the tyrosine crystals (Rajatasilpin, et al, 1961). However, the excretion of these crystals in the urine was not persitent:

It was found only on the second day of the experiment and was not found even fed the monkey for the other week.

The toxicity of Niang bean extract on albino rats were also studied in the present studies. Besides the increasing of amorphous particles, red cells and white cells in urine, no significant changes were observed. This finding was in accordance with the previous works done in this species of animal (Rajatasilpin et al. 1961).

The results in the present studies showed that the rosettes of needle shaped crystals were found in urine of a mouse after feeding with Niang bean extract. These crystals were very similar to those that were prepared from a commercial L-djenkolic acid. Only one out of 22 mice excreted these crystals. In what condition the crystals may form is not known. This may depend on the following factor: animal species, the proper body pH, the capability of enzymes for the formation of the crystals and some other unknown factors. Because the formation of the crystals in the urine is uncertain, therefore not everyone who eats the Niang beans must develop such manifestation.

Toxicity of Niang extract in the other animal species has been investigated. Rabbits, guinea pigs and dogs were fed with these beans; no remarkable changes have been observed in these animals (Mreyen, 1941, Mokasmith, 1961).

Histological examination of the kidneys in mice and rats has been studied in the present study; kidneys showed cloudy swelling and hydropic degeneration with haemorrhage in the tubules of mice. Some mice showed progressive necrosis of tubular cells with cloudy swelling. The results in rats were very similar to those of mice, i.e., degeneration of kidney especially tubular cells with cloudy swelling and haemorrhage. Mreyen (1941) fed beans to one monkey and to a few rabbits and guinea pigs. Tissues of animals killed 6 to 12 hours after feeding showed a leukocytic infiltration of the peripelvic renal connective tissue and exfoliation and obliteration of some tubules. Biopsy made during renal decapsulation on 2 anuric patients showed swelling and exfoliation of the tubular epithelium in one. In the other, there were a few leucocytes in the glomeruli and acute haemorrhagic inflammation in the perirenal tissue.

All histological studies described above indicated that djenkolic acid could cause degeneration with haemorrhage of the tubular cells in those animals. These pathological changes may be due the laceration of renal tissue by the sharp crystals of djenkolic acid as proposed by Van Veen and Hyman (1933). These could also be due to an allergic reaction or other irritating effect on the renal parenchyma, on the capillaries, on the cells lining the urinary tract, or even on the renal nervous system. Serial needle biopsies of the kidney might be informative in this particular question.