

A NOTE ON THE CHEMICAL  
EXAMINATION OF NIM BLOSSOMS  
(*MELIA AZADIRACHTA*)

RESULTS of chemical examination of nim blossoms have been recorded by us in a previous publication.<sup>1</sup> Without making any reference to this work, Subramanian and Rangaswamy in a subsequent communication<sup>2</sup> claimed the separation of certain substances from the blossoms, which are not well defined and some of which appear to form only intermediate stages in the isolation of the various products reported by us. Thus the golden yellow oil reported by them was obviously a crude form of the sesquiterpene derivative. In this communication the isolation of two bitter principles (yield of one, 1.3 per cent.) from the blossoms has been reported. As the blossoms in the Delhi area are not bitter in taste, and no bitter principle was found to be present in any of the fractions derived from the alcoholic extract, it was considered advisable to check up the earlier results by following up the method adopted by Subramanian and Rangaswamy<sup>2</sup> in the working of the blossoms. No bitter principle could, however, be isolated from the blossoms in this area. On the other hand all the products isolated before<sup>1</sup> were identified in this working also.

The alcoholic extract from 2 kilos of the blossoms was repeatedly extracted with ether. From the ether-insoluble fraction, nimbicetin was obtained after acid hydrolysis.<sup>1</sup> The ether solution was then repeatedly extracted with 5 per cent. caustic soda solution. The residue (Ca. 35 gms.) after complete removal of the solvent from the well-washed and dried ether layer, was a thick, brownish, oily liquid with the characteristic pungent smell of the essential oil. It was digested with petroleum ether when a very small quantity of waxy white material was left behind. The petrol ether solution was repeatedly partitioned with 70 per cent. dilute alcohol, when a comparatively small quantity went into the alcoholic layer. The semi-solid, light brown gummy residue (Ca. 1.5 gm.) from the alcoholic layer had the characteristic smell of the essential oil and was not bitter to taste. The acrid pungent taste of the essential oil could, however, be detected with its alcoholic aqueous emulsion. On further digestion with hot petroleum ether, the residue yielded some more steam volatile product from the petrol ether digestive. The residue from petrol ether digestion formed a brownish yellow solid (Ca. 0.06 per cent.) with the smell of essential oil. This residue which would correspond to the bitter, marked "C" by Subramanian and Rangaswamy, was actually not bitter to taste. It was, moreover, optically inactive in contrast to the nimbidine series of bitters isolated from the various parts of the plant.<sup>4</sup> This residue was then subjected to steam distillation when a minute quantity distilled over. The resinous residue was a brittle, light brown solid, sparingly soluble in cold alcohol. It was hydrolysed with 5 per cent. cold alcoholic potash for 20 hours and worked up in the usual manner. Neither the neutral nor

the acidic component of the hydrolysates was bitter to taste.

The residue from the petrol ether—a brownish pasty mass with the pungent taste of the essential oil—when distilled with steam, yielded the major fraction of the essential oil (0.3 per cent.). The waxy, solid residue from the distillation had no marked taste, and on saponification with 20 per cent. alcoholic potash gave nimbosterol and nonakosane from the unsaponifiable fraction. The acidic components were not investigated further as the constituent acids had been exhaustively studied.<sup>1</sup>

The alkaline extract from the ether-soluble portion of the original alcoholic extractive was acidified with dilute HCl, and the greyish-brown precipitate filtered off. The aqueous filtrate was distilled, when it yielded the product corresponding to the acidic fraction of the essential oil.<sup>1</sup> The acidic precipitate on repeated purification with solvents, yielded a small quantity of a mixture of fatty acids which were not followed up further in this working.

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January 2, 1948.

1. Mitra, Rao, Bhattacharya and Siddiqui, *J. Sc. Ind. Res., India*, 1947, **6 B**, 19. 2. Subramanian and Rangaswamy, *Curr. Sci.*, 1947 **16**, 182. 3. Siddiqui and Mitra, *J. Sc. Ind. Res. India*, 1945, **4**, 5. 4. Mitra, Rao and Siddiqui., *Proc. Indian. Sc. Cong.*, 1947 (Adv. Abs. Chem.) **73 a** Mitra and Siddiqui, *Ibid*, 1948.

EFFECT OF SUPPLEMENTATION  
WITH TAMARIND AND CHILLI ON  
THE GROWTH OF YOUNG RATS ON  
A POOR-SOUTH-INDIAN-RICE DIET

ALTHOUGH millions of people have lived for generations, on a poor rice diet composed mainly of rice, the growth response and fertility of rats on experimental diets composed of the same major components have been repeatedly observed to be disappointing.<sup>1-4</sup> This apparent discrepancy is either due to the unsuitability of the rice diet to rats or, possibly, the absence of some supplement which forms a part of the normal human diet, but which is excluded from the experimental diet. A significant omission made in the formulation of the experimental rice diet is with regard to tamarind and chilli, which are invariably added to the rice diet as consumed in South India. As these two ingredients are normally considered to be unimportant in evaluating the food value of experimental diets, it is of interest to determine whether their incorporation would make any difference in the response of the rat to the Poor-South-Indian-Rice-Diet.

Twelve rats from our stock colony were weaned, eighteen days after birth, at the weight of 28 gms., and placed on the rice diet plus 10 c.c. of 10 per cent. Klim milk each till they

weighed about 40 gms. This preliminary procedure has been found to be desirable to acustom the rats to the rice diet. The rats were divided into two groups of six each with equal number of littermates of the same sex. The first group received a poor rice diet of the following percentage composition:

Polished rice, 78.5; tur dal (*Cajanus indicus*) 5.0; common salt, 0.3; non-leafy vegetables, 8.2; leafy vegetables, 2.1; whole milk powder (Klim), 0.9; crude groundnut oil, 5.0.

This diet does not differ materially from the conventional rice diet used by most of the earlier workers. The rice, dal, vegetables and salt were mixed together and cooked with three to four times its volume of water. The crude groundnut oil was mixed with the cooked diet. The milk powder was made into a 10 per cent. solution and fed to the rats separately.

For the second group, the diet was prepared in the typical South Indian style by using tamarind, chilli and extra salt to taste which together made up 2 per cent. of the diet replacing an equal proportion of rice in the above composition. An aqueous extract of the ripe tamarind as prepared in the household and dry chilli powder was used. Extra salt (0.2 per cent.) was also added so as to correspond to the normal diet.

The difference between the two diets in regard to essential constituents (protein, fat, carbohydrate, calcium and phosphorus) is almost negligible.

The growth rate of the animals over a period of 15 weeks are presented in Table I. It was observed that the animals in the second group took slightly longer to get adapted to the tamarind and chilli. The animals receiving tamarind and chilli as supplement were distinctly more active than those on the rice diet alone. In both the groups, there was shedding of hair but this was less pronounced in the tamarind group than in the control. There was no mortality in either of the groups during the experimental period. After that period, the animals were mated. Some of the animals of the tamarind group gave birth to litters, whereas none of the control group has so far done so in spite of over two months of pairing. The related observations will be continued with the succeeding generations.

TABLE I  
Poor Rice Diet

Sex	Initial wt. (average)	Final wt. (after 15 weeks) (average)	Average food intake (gm. dry wt.) per day	Average weekly increase gm.
M ..	40.5	105.3	8.2	4.34
F ..	40.2	94.7	8.07	3.61
Poor rice diet supplemented with tamarind and chilli				
M ..	40.8	123.0	8.57	5.5
F ..	39.3	110.0	8.33	4.7

The average food intake of the rats receiving supplement of tamarind and chilli was only slightly more than that of the animals on the rice diet. The increase in growth of the former was distinctly out of proportion with the extra food intake.

The above is only a preliminary note indicating the importance of two food components which had not been considered to be of any nutritional significance. Further work extending the above findings and designed to throw light on the mechanism of action is in progress.

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1. Aykroyd *et al.*, *Indian J. Med. Res.*, 1937, 24, 1093. 2. *Idem*, *Ibid.*, Memoirs No. 132. 3. Eleanor Mason *et al.*, *Ibid.*, 1945, 33, 219. 4. "Report to the Vanaspati Research Committee," Ministry of Food (unpublished).

#### FACTORS AFFECTING THE NUTRITIVE VALUE OF SOYA BEAN PROTEIN

It is now well known that the nutritive value of soya bean protein which is low in the raw state is appreciably raised by suitable heat treatment.<sup>1-5</sup> Johnson *et al.*<sup>6</sup> attribute this difference in nutritive value to the presence of a nitrogen-sulphur complex in the raw protein which, they believe, cannot be utilised and that heat treatment makes it available for tissue-building purposes. This has also been confirmed by later work.

Recently Ham *et al.*<sup>7-8</sup> found that a trypsin inhibitor in the raw bean interferes with the utilisation of protein. Melnick *et al.*<sup>9</sup> have also suggested the mechanism for the action of this inhibitor. Experiments carried out in this laboratory have led to the following interesting findings with regard to the role of the trypsin inhibitor and other possible factors affecting the nutritive value of soya bean protein.

- (1) The removal of the tryptic inhibitor by acid extraction of raw soya bean meal raises the growth-promoting value of the protein from 1.2 to 1.4, while autoclaving raises the value to 1.9.
- (2) The tryptic inhibitor being heat-labile, all types of heat treatment must be expected to increase the nutritive value of the protein to about the same extent; but only wet heating and particularly autoclaving has been found to have a beneficial effect on the nutritive value.
- (3) Germination of the soya bean increases the nutritive value of the protein; but the concentration of the tryptic inhibitors in the raw and the germinated beans remains the same.

The above evidence, as also the observations made by Riesen *et al.*<sup>10</sup> on the digestibility of