

A METHOD OF SECTIONING THE
GAMETOPHYTES OF SOME
LIVERWORTS AND PTERIDOPHYTES

CONSIDERABLE difficulty is often experienced in cutting smooth sections of many terrestrial and sub-terrestrial gametophytes. The following method was evolved to overcome this difficulty:—

(1) Fix the material in Formalin-acetic-alcohol or Nawashin's fluid. Wash with 50 per cent. alcohol in the former case and water in the latter.

(2) Run up to 70 per cent. alcohol and remove as much of the sand as is conveniently possible by means of a camel-hair brush.

(3) Treat with 5 per cent. HF in 70 per cent. alcohol—3 to 5 days—keeping the material in specimen tubes having a thick coating of paraffin on the inside. This is done simply by dipping the vial 2 to 3 times in melted paraffin and pouring it out.

(4) Wash out HF by repeated changes with 70 per cent. alcohol.

(5) Run up through the alcohol and xylol series and imbed in paraffin as usual.

(6) Soak the blocks in water for a week and cut at desired thickness—6 microns or above.

The cell structure was not damaged in any way for morphological or embryological studies. Good staining was obtained with both Safranin-Fast Green and Iron-Hæmatoxylin. The HF merely dissolves the finer sand particles that persist after the preliminary cleaning in step 2.

It is hoped that other botanists will try this method and report if cytological details are appreciably affected by the action of HF.

I am greatly indebted to my teacher, Dr. P. Maheshwari, who suggested the method for a trial.

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BITTER PRINCIPLES OF THE
NEEM OIL

IN connection with the note on this subject by Siddiqui, recently published in *Current Science*,¹ attention may be drawn to a paper with the above title published in the *Indian Journal of Pharmacy* by Murti, Rangaswami and Seshadri² in October 1940. This paper seems to have escaped the notice of the author of the note in *Current Science*. In it, Murti *et al.* have clearly anticipated the mild methods of continuous extraction with warm alcohol and fractional separation of the crude extract by the use of organic solvents and they have employed these methods in the examination of the oil and the oil-cake. The most important observation was regarding the nature of the solid deposit obtained by allowing the oil to stand for a long time. This contained all the quantity of the bitter principle originally present in the oil. It could be separated into two definite fractions A and B using benzene, and substances of the same characteristics were obtained from the three sources mentioned above, *viz.*, the oil, the oil-cake and the deposit. Though they could not be obtained in a crystalline form with well-defined melting points and hence could not be given definite names, they seemed to have constant composition and properties. They were both sulphur-free, non-glucosidal bitter substances devoid of any odour. They were insoluble in water, soluble in alcohol and had reducing properties. Substance A, which had the empirical formula $C_5H_7O_2$, was soluble in benzene, whereas B with the empirical formula $C_4H_7O_2$ was insoluble in that solvent. When tested on earthworms and fresh-water fish, they exhibited no toxic properties in a concentration of 1 in 1000.

The components now described by Siddiqui seem to be, in general, of the same nature as the substances A and B. The two minor components, nimbin and nimbinin, are described as being sulphur-free, neutral and water-insoluble just like A and B. There is, however, some difference since nimbin and nimbinin are said to be crystalline compounds with sharp melting points. Further, nimbin is

reported to have a higher carbon content, 66.6 per cent. as against 61.3 and 54.3 per cent. obtained for A and B respectively. The major bitter component nimbidin, however, is said to contain probably sulphur also and thus differs from substances A and B, though it is also amorphous. In the earlier work of Murti *et al.* it was stated that though the crude bitter solid contained sulphur, it disappeared in the course of purification and hence they felt that the sulphur-containing impurity was very small in amount.

It may perhaps be right to conclude that the bitter principles of neem oil are of complex nature and not unlike the active components of such well-known bitters as quassia and gentian.

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¹ Siddiqui, *Curr. Sci.*, 1942, **11**, 278.

² Murti, Rangaswami and Seshadri, *Ind. Jour. Phar.*, 1940, **2**, 206.

OMISSION of the reference is regretted. It was due to the fact that the paper in question was published in a recently started *Journal of Pharmacy* and came to my notice only through the chemical abstracts¹ after the publication of the note on the bitter principles of Neem oil. Due reference would have been made to the work in the detailed communication on the subject.

Extraction of Neem oil with alcohol and isolation of water insoluble, neutral and acid

bitters from the alcoholic extract, with the help of dilute alcohol and other solvents, has been already referred to by Dymock.² The results obtained there or now by Seshadri *et al.* show the limitations of this mild method. The success of the procedure employed in the isolation of Nimbin and other products communicated in the note in *Current Science*³ is demonstrated by the uniformity of the isolated products and their yields. The details of this process will be dealt with in a subsequent communication.

With regard to the substances A and B obtained by Seshadri *et al.*, it will be noted that they are amorphous powders which decompose at 115 and 110-115° respectively. B, moreover, melts at 72°, prior to decomposition at 110-115°. Nimbin and Nimbinin, which are definite crystalline substances melting at 205° and 192° respectively, cannot, therefore, be confused with either of them. As far comments on Nimbidine or on the relationship between the active components of Nim and gentian or other bitters, it will be more appropriate to discuss them at a later stage of the investigations, which are now in progress.

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¹ *Ame. Chem. Soc. Abst.*, May 1942, p. 2685.

² *Pharmacographia India*, 1890, **1**, 327.

³ *Curr. Sci.*, 1942, **11**, 278.