EXTRACTION OF SAP FROM PLANT TISSUES.

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It was first observed by Andre (*Compt. Rend.*, 1906, **142,** 106) that successive portions of juice expressed from fresh plant parts differ from one another in their chemical composition. Marie and Gatin ("Determinations Cryoscopiques effectuees sur les sucs vegetaux", 1912) noticed a progressive increase in concentration in their sap studies, but contented themselves with assuming the mixture of different pressings to represent the juice originally present in the tissue. Dixon and Atkins (*Sci. Proc. Roy. Dublin Soc.*, N.S., 1913, **13,** 422) recognised that this variation in the concentration of sap would militate against its adoption in physiological studies and suggested that tissues should be treated in some manner prior to extracting the fluid. Gortner, Lawrence and Harris (*Biochem. Bull.*, 1916, **5,** 139) advocated a similar procedure but found that the concentration of the successive pressings varied with different species. Recently, one of us (A.V.V., M.Sc. Thesis, Univ. Madras, 1929) observed an increase in density in the different pressings from the fresh leaves of sandal (a plant parasite), mahogany and a few other species.

It is thus evident that untreated fresh tissues cannot be employed in physiological studies. Dixon and Atkins (*loc. cit.*) therefore compared several possible methods of pre-treating the tissues and concluded that exposing them to very low temperature such as that of liquid air renders the cell walls permeable to cell contents, ensures easier expression and yields a juice which is richer in composition and more uniform in character than those obtained by other methods. Various modifications of this technique have subsequently been introduced to secure easy expression of cell contents; for instance, ice and salt mixture by Gortner and Harris (*Plant World*, 1914, **17,** 49) and solid carbon dioxide by Harvey (*J. Agric. Res.*, 1918, **15,** 83) and others (*vide* Meyer, *Plant Physiol.*, 1929, **4,** 103). Dixon and Atkins also tried the efficacy of toxic vapours such as toluene and chloroform for pre-treating plant tissues. They found that by this method a sap somewhat rich in solutes could also be obtained. Such a procedure did not, however, check enzyme activity of the tissue during the protracted treatment which lasted for nearly 36 hours. Goldsmith and Smith (*Colorado Coll. Pub. Sci. Ser.*, 1926, **13,** 13) treated tissues for the same period with chloroform and refrigerated the material to minimise such enzymic action.

Although a number of methods have been suggested, it does not, however, appear that any comparison has so far been made of the

different methods of treatment using the same kind of tissue material for this purpose. It is not always possible that the same method can be adopted. Moreover, some of the methods require costly equipment which may not be readily available in most laboratories.

In the course of an investigation on tissue fluids of healthy and spiked sandal, a preliminary comparative examination was made on leaf tissues exposed both to liquid air and toluene. In the latter case, the treatment was followed by refrigerating the tissues overnight in ice-salt mixture maintained at −16 to −20°. The controls were preserved in an ice chamber maintained at −2°. The details of the treatment and expression of sap were the same as those described in a previous communication (A.V.V., *J. Indian Inst. Sci.*, 1929, **12A,** 295). In each case, the concentration was determined by the thermoelectric method of Dixon and Atkins in terms of depression of freezing point of juice and reckoned as osmotic pressure in atmospheres (*J. Indian Inst. Sci*, 1928, **11A,** 103).

TABLE I.

Sap concentration of leaves under different treatments.
(*100 grams of tissue employed in each case.*)

Leaf specimens from	Yield of sap in Grams with			Osmotic Pressure of sap in Atmospheres with		
	No treatment	Toluene	Liquid Air	No treatment	Toluene	Liquid Air
Healthy sandal ..	34·2	68·4	74·9	9·43	15·52	17·80
Spiked sandal ..	31·1	58·7	62·6	9·54	16·11	19·49
Mahogany ..	24·2	61·1	66·4	4·86	8·90	11·79

It is clear from this that liquid air treatment leads to a larger yield and richer sap than by other methods. One of us (*loc. cit.*) has elsewhere confirmed the observations of Dixon and Atkins regarding the uniformity in composition of successive pressings from such tissues. Toluene comes next in order, while the untreated one comes last.

It appeared necessary to examine alternate methods to replace liquid air treatment, when this is not available or where only a comparative study is being made. Thus, Narasimhacharya and Sastri (*J. Indian Inst. Sci*, 1931, **14A,** 1) used toluene in their studies on phaseolus while Goldsmith and Smith (*loc. cit.*) employed chloroform. In view of the fact that this choice has been rather arbitrary, it was considered desirable to have an idea of comparative efficiencies of the different cytolysing agents for the purpose. Some studies were carried out therefore with sandal leaves after treatment with toluene, chloroform

or ether. In all cases, the tissues were refrigerated overnight in ice-salt mixture at −16° in glass stoppered bottles. The yields of sap thus obtained have been presented in Table II.

TABLE II.

Yield of sap (in grams) from 100 grams of sandal leaves.

Quantity of reagent used in c.c.	Ether	Chloroform	Toluene
1	..	58·0	46·9
2	49·5	58·7	52·8
5	53·8	59·0	53·0
7	55·3	59·2	53·3

The solid contents of the above saps have been given in Fig. 1.

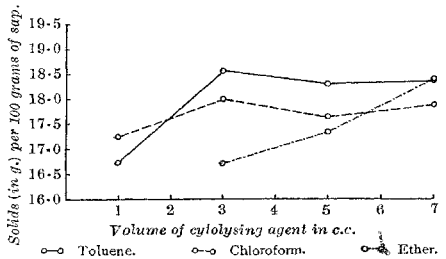

Fig. 1.
Total Solid Content of Expressed Sap.

It may be seen from the above that while chloroform gave the largest yield of sap, the juice obtained with toluene was the richest in total dissolved matter. Treatment with ether does no yield satisfactory results. It may be argued that there is not significant difference between toluene and chloroform and that both are equally efficient. In sap studies the concentration of the expressed fluid is even more important than the total yield, since in the latter even debris from broken cells may be present. From this point of view, toluene would appear to be preferable to chloroform.

The following (Table III) were the results obtained with the expressed juice from the leaf tissues of ragi (*Eleucine coracana*).

TABLE III.

Dissolved matter in ragi leaf sap.

(*Expressed in grams for 100 c.c. of sap.*)

Cytolising agent with quantities in c.c.	Total solids
Toluene (5) ..	18·81
Chloroform (5) ..	18·52
Ether (10) ..	18·36

It may be seen from the above that toluene was slightly better than chloroform. It was therefore employed in our subsequent studies. It may also be mentioned here that in the cryoscopic or total solids determinations with the several treatments, corrections should be made for the solubility of the cytolising agent in distilled water to obtain reliable data.

SUMMARY AND CONCLUSIONS.

1. A comparison of the different methods of sap extraction has shown that exposure of plant tissues to liquid air prior to extraction, results in securing a juice which is richer and more uniform in composition than those with cytolysing agents. The latter are, however, more convenient to handle and can be employed, when fairly accurate estimates are required or when juices from a number of similar materials are being compared.

2. Among the cytolysing agents that were tried, toluene and chloroform were found to be the most satisfactory. The former yielded however, a slightly more concentrated sap than the latter.

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