# THE USE OF ESSENTIAL OILS IN HISTOLOGIC PRACTICE

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A series of essential oils such as oil of cloves, bergamot, camphor, and others are used in various basic histological procedures principally for the purpose of extracting the celloidin and clearing the sections. This is especially important before many histologic and bacteriologic stains can be used.

Cajuput terpene oil and oil of cloves, which are so widely used for these purposes, are expensive and difficult to obtain.

In our search for substitutes we employed for experimental histologic procedures the essential oils of the following plants: lemon sorghum (Cymbopogon citratus), eugenol basilik (Ocimum gratissimum), lemon eucalyptus (Eucalyptus citriodare), eucalyptus (Eucalyptus viminalis), pink geranium (Pelargonium roseum), patchouli (Pogostemon patchouli) and melalevka (Melaleuca alternifolia).•

## EXPERIMENTAL METHODS

Extractions from celloidin sections were conducted in the following manner. The section is removed from the water on to a glass slide, smoothed, and dried with a piece of filter paper. After the celloidin is extracted from the slice, the glass slide is rinsed out first with absolute, and then with 96% alcohol. Then it is rinsed with water and stained according to various methods. Celloidin can also be removed by dehydrating the section in 96% alcohol and then adding it to a jar with the essential oil. After rinsing with alcohols and water, we can then stain these sections by the same procedures employed on the frozen slices.

After removing the celloidin, we stained the sections with hematoxylin-eosin, picrofuchsin and Mallory. The sections subjected to the action of the essential oils retained their capacity to stain, the color being retained for as long as $2^1/_2$ months.

Celloidin was entirely removed within 7 to 15 minutes by the oils of lemon sorghum, eugenol basilik, lemon eucalyptus and pink geranium, the sections clearing at the same time. Celloidin was removed most rapidly by oil of pink geranium (7 minutes).

Also investigated was the possibility of using various volatile oils in staining nerve cells. To test this, material from monkey brains was taken, fixed in 96% ethyl alcohol, and imbedded in celloidin, as in common procedures for staining nerve tissues. We used 0.1% toluidine blue in aqueous solution followed by rinsing in several portions of 96% alcohol. Instead of clearing the slices with the usual cajuput oil, we used oils of melalevka, eugenol basilik, patchouli, pink geranium and lemon eucalyptus. As controls, a portion of the sections was differentiated with cajuput oil. Later, as with clearance with cajuput oil, the essential oils were washed out with pure xylene and the sections were covered with Canadian balsam.

• These oils were obtained from the Sukhumi essential oils zonal station where their properties were first studied in detail by the director of the biochemical laboratory of the station, A. A. Pravdolyubov, and then turned over to us for further experimentation.

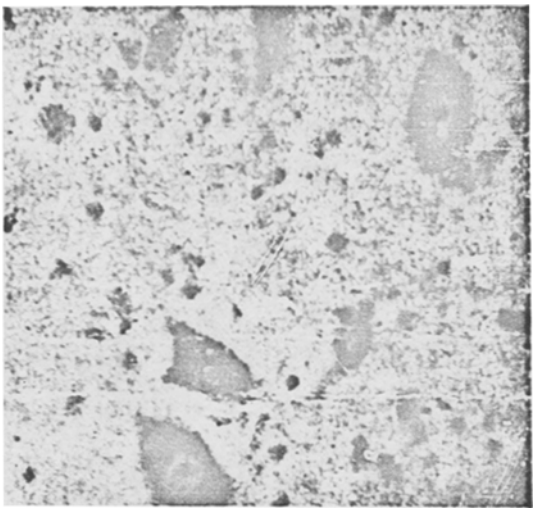

Fig. 1. Staining of the nerve cells from spinal cord anterior horn with toluidine blue. Cleared with patchouli oil. Objective 40, ocular 7.

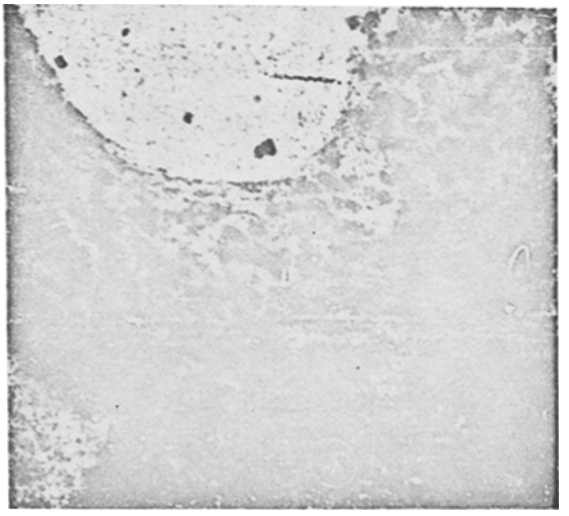

Fig. 2. Pulmonary tissue stained after van Gieson. Celloidin extracted with lemon sorghum oil. Objective 20, ocular 7.

## EXPERIMENTAL RESULTS

The nerve cells and especially their chromatophilic substances stained best after the sections had been cleared with melalevka and patchouli (Figs. 1 and 2). Both sections were indistinguishable from the controls. The chromatophilic substance stained intense blue with a lilac sheen. The nuclei of the nerve cells, with the exception of the nucleoli and the fibrous brain structures, remained unstained. The glia cells were stained in the usual manner. Oil of melalevka was superior to patchouli oil in that the sections cleared within 1-2 minutes, as against 7-10 minutes for patchouli.

The investigations with the other essential oils gave less favorable results. When oils from eugenol basilik, pink geranium and lemon eucalyptus were used, clearing occurred within 7 to 15 minutes. However, only a portion of the nerve cells resembled the control sections in acquiring the blue-lilac sheen. In a number of the cells, the chromatophilic substance, as well as the protoplasm and some nucleoli, stained green. At the same time the contours of some of the cell elements remained indistinct. The entire slice stained diffusely a light green tone. This staining defect was most pronounced with eugenol basilik oil. When the sections were treated with pink geranium, only individual nerve cells acquired the green shade. In sections with lemon eucalyptus, the nerve elements stained well and only occasional tissue and glial cells took on the green color. When the last three essential oils were used, the celloidin was either partially or fully dissolved at the moment of full clearing, depending on the thickness of the section.

It should be observed that all the volatile oils studied by us are readily removable with ethyl alcohol, but the pink geranium and eugenol basilik oils could not always be fully washed out with xylene. The possibility is not excluded that this reflects on the quality of the stain after the preparation is stored.

Thus, instead of using oil of cloves for the extraction of celloidin from the preparations, we found that the following volatile oils could be used without lowering the quality of the sections: eucalyptus (Eucalyptus citriodare), lemon sorghum (Cymbopogon citratus), eugenol basilik (Ocinum gratissimum) and pink geranium (Pelargonium roseum).

The essential oils of melalevka and patchouli appear to be the best substitutes for cajuput oil in the staining of nerve cells.

## SUMMARY

Clearing of microscopic sections as well as extraction of celloidin from them can be successfully accomplished with the use of such essential oils as Cymbopogon citratus, Ocimum gratissimum, Eucalyptus citriodare, and Pelargonium roseum instead of the usual oil of cloves. For differentiating and staining nerve cell preparations, good results have been obtained with the use of the essential oils of Melaleuca alternifolia and Pogostemon patchouli instead of cajuput terpene oil.