Characterization of the Mutagenic Component of *Siling Labuyo*

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The ethyl acetate fraction of siling labuyo *was found to be mutagenic and clastogenic. Thin layer chromatography on silica gel plates using chloroform and absolute ethanol (9:1) gave five spots. Only spot no. 5 was shown to be genotoxic as determined by the micronucleus test. The ultraviolet absorption spectrum, proton NMR spectrum, low resolution mass spectrum, and HPLC data showed that spot no. 5 was not capsaicin. The mutagen in* siling labuyo *is not capsaicin, contrary to the claim in the literature. Further studies are necessary to determine the exact structure of spot no. 5.*

INTRODUCTION

Expressions from the mature fruit of *siling labuyo (Capsicum frutescens)* have been shown to induce the formation of micronucleated polychromatic erythrocytes indicating mutagenic and clastogenic properties (1).

Capsaicin, which is present in fruits of the *Capsicum* species, has been suspected to be the mutagenic component (2).

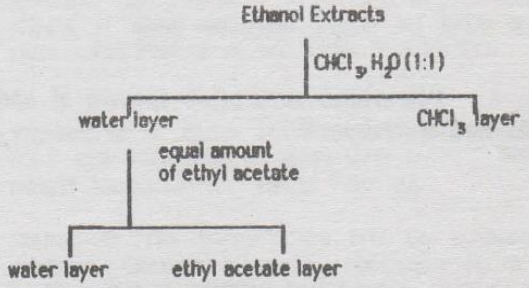
This work was undertaken to find out if the mutagenic component of *siling labuyo* is indeed capsaicin.

MATERIALS AND METHODS

Ripe and mature fruits of *siling labuyo* were obtained from the Divisoria market. These were air-dried before grinding in a corn mill.

About 100 g of the ground sample were extracted with hexane for 6 h using the Soxhlet extraction method, and then air-dried overnight. The air-dried samples were extracted with 95% ethanol for 6 h.

The ethanol extracts were fractionated (**see diagram next column**).



After fractionation, the various layers of water, ethyl acetate, and chloroform were dried under vacuum. The residues were taken up in dimethylsulfoxide (DMSO) and tested for genotoxicity to bone marrow cells using the micronucleus test (3).

The ethyl acetate residue was subjected to thin layer chromatography (TLC) on silica gel/UV 254, using chloroform:absolute ethanol (9:1) as solvent. Capsaicin, obtained from Sigma Chemical Company, was run together with the ethyl acetate fraction.

The five spots obtained in the thin layer chromatogram were scraped and pooled together. These were eluted with 95% ethanol. Ethanol was removed under vacuum. The residues were tested for genotoxicity using the micronucleus test.

The spot that showed mutagenic activity was used for the determination of absorption spectrum, mass spectrum, NMR spectrum and HPLC. A capsaicin standard was run simultaneously.

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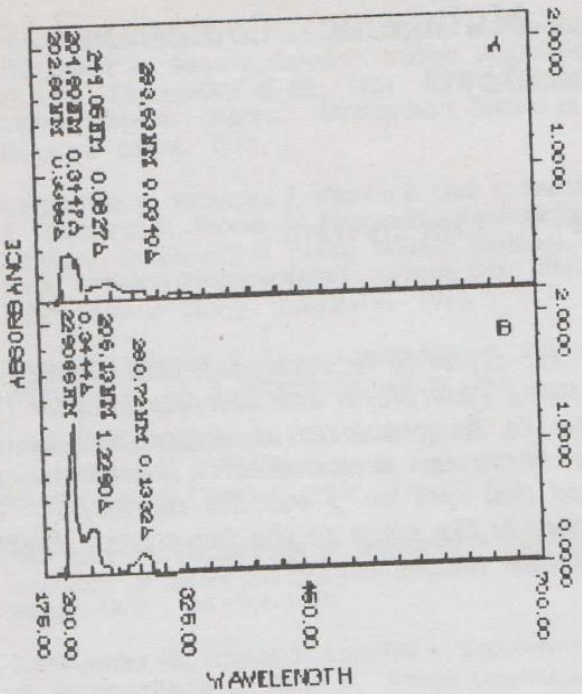

Fig. 1. Ultraviolet absorption spectra of spot no. 5 (A) and capsaicin (B).

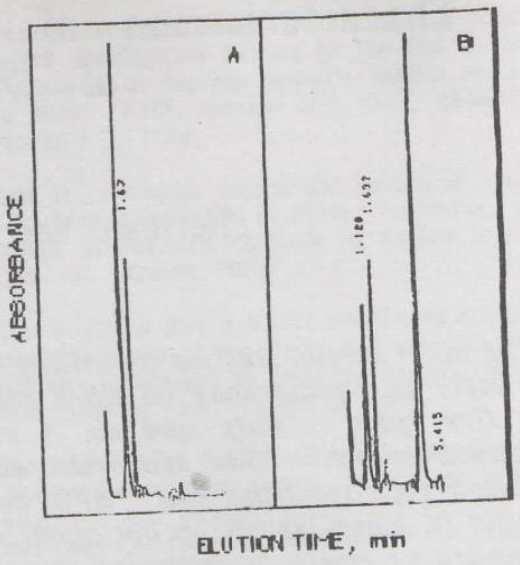

Fig. 2. High pressure liquid chromatogram (HPLC) of spot no. 5 (A) and capsaicin (B).

RESULTS AND DISCUSSION

The ethyl acetate extract produced 13.00 ± 0.97 micronucleated polychromatic erythrocytes per thousand in bone marrow cells of experimental mice, while the chloroform extract formed only about half of that (6.13 ± 0.83). The ethanol extract gave a value of 9.00 ± 1.23 while the water extract produced as much as DMSO, which was the solvent control (4.70 ± 0.98). The ethyl acetate fraction was therefore said to exhibit mutagenic and clastogenic activity since the formation of micronucleated polychromatic erythrocytes was more than twice the solvent control.

The ethyl acetate fraction and a capsaicin standard were simultaneously subjected to thin layer chromatography on silica gel/UV 245, using chloroform and absolute ethanol as solvent (9:1). Five spots were obtained from the ethyl acetate fraction. Capsaicin had the highest R_f value of 0.80, followed by spot no. 5, with a value of 0.63. Spots 2, 3, and 4 had R_f values of 0.08, 0.18, and 0.50, respectively. Spot no. 1 did not move at all.

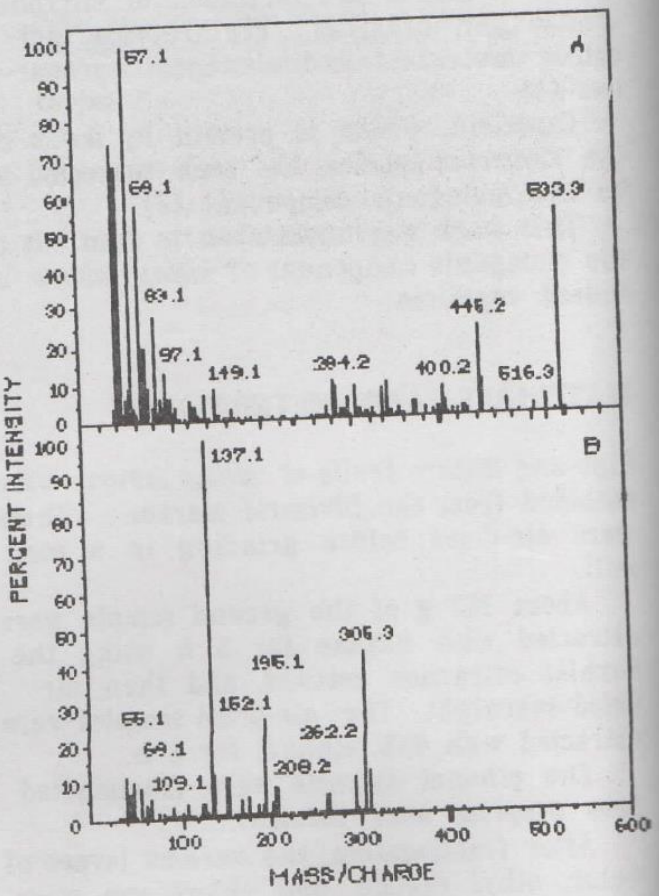

Fig. 3. Low resolution mass spectra of spot no. 5 (A) and capsaicin (B).

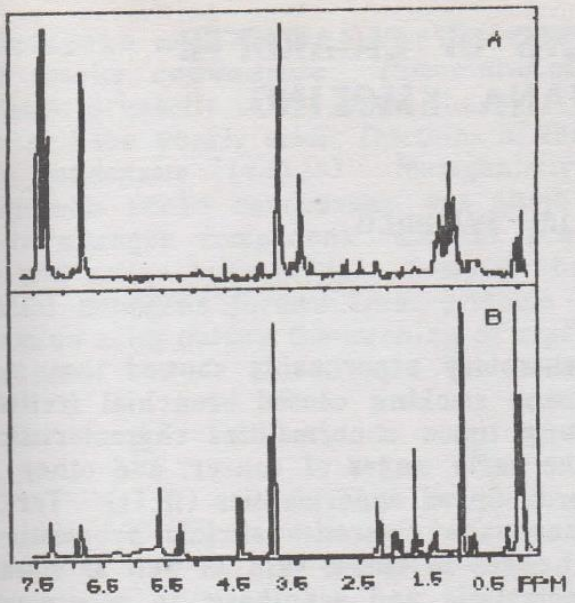

Fig. 4. Proton NMR spectra of spot no. 5 (A) and capsaicin (B).

The micronucleus test revealed that only spot no. 5 was genotoxic, forming 13.11 ± 1.67 micronucleated polychromatic erythrocytes per thousand cells, as compared to a value of 5.81 ± 0.82 for the solvent control. The other spots (no. 1, 2, 3, and 4) gave values of 7.11 ± 0.78, 7.67 ± 0.87, 7.55 ± 0.88, and 9.33 ± 0.82, respectively.

The comparative and simultaneous ultraviolet spectrum, mass spectrum, NMR spectrum, and HPLC runs of the capsaicin standard and residues from the genotoxic spot no. 5 gave nonidentical results (Figs. 1-4).

From the foregoing results, it can be concluded that the mutagenic spot no. 5 obtained from the ethyl acetate fraction of *siling labuyo* is not capsaicin. ※

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