

**SMILAX ZEYLANICA LINN.—A NEW SOURCE OF DIOSGENIN**

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SAPOGENINS are commercially important due to their extensive use as precursors of many pharmacologically active steroids<sup>1</sup>. The most important economic saponin is diosgenin, commercial extraction of which is almost entirely from species of *Dioscorea*. With the increasing demand for diosgenin, exploitation of previously untapped wild sources is becoming more important. The genus *Smilax* of the family Liliaceae has been used medicinally since long and it furnishes the drug sarsaparilla<sup>2</sup>. Previous phytochemical investigations on *Smilax* for saponin revealed the presence of sarsasapogenin in *S. aspera*<sup>3</sup>, smilagenin in *S. ornata*<sup>4</sup> and diosgenin in *S. china*<sup>5</sup>, *S. parvifolia*<sup>6</sup> and *S. perfoliata*<sup>7</sup>. But no attempt has so far been made on *S. zeylanica*, an indigenous wild medicinal species. The present investigation was undertaken for the analysis of saponin from root and leaf as well as from *in vitro* callus of *S. zeylanica*.

Roots and leaves of *S. zeylanica* were harvested from the experimental garden. Callus was developed from young shoot segments growing on Murashige and Skoog's basal medium<sup>8</sup> supplemented with indole butyric acid (1.0 mg/l) and kinetin (0.5 mg/l).

The materials were dried, powdered and extracted with petroleum ether after hydrolysing in 30% v/v hydrochloric acid. The extract was examined for the presence of saponin by thin-layer chromatography (TLC) using the solvent system chloroform: benzene (90:10). Liebermann-Burchard reagent<sup>9</sup> (acetic anhydride: conc. sulphuric acid: ethanol: 1:1:10) was used for the detection of saponin on chromatogram. Gas liquid chromatography (GLC) was done with a U-shaped steel column (50 cm × 5 mm) and the stationary phase was 10% UCW-982, 80-100 WAW-DMCS. The column temperature was 240°C and N<sub>2</sub> the carrier gas being kept at a pressure 3 kg/cm<sup>2</sup>. Authentic samples of smilagenin, sarsasapogenin and diosgenin were used.

Presence of diosgenin both in root and leaf as well as in the callus was recorded through both TLC and GLC analysis.

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**NEW TECHNIQUES TO DETERMINE POLLEN VIABILITY OF *CALOTROPIS GIGANTEA* R. Br.**

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*IN VITRO* germination is the most commonly used test for pollen viability. A major limitation of this test is the difficulty in finding out percentage germination in taxa having pollinial apparatus, since the pollen grains remain together forming a single mass, called the pollinium. Saoji and Chitale<sup>1</sup> observed that the germination percentage in *Calotropis gigantea* R. Br. (Asclepiadaceae) could not be calculated, for the exact number of pollen tubes and the number of cells involved therein could not be ascertained. Similar difficulties were encountered in *in vitro* pollinial germination in some members of Asclepiadaceae<sup>2-5</sup>.

Two methods found to be successful in determining pollen viability of *C. gigantea* are reported.

*In vitro* germination method

In this method mature and intact pollinia dissected out from flowers just opened of *C. gigantea* were used. The hanging drop technique was followed. All germination studies were carried out at room temperature (25 and 31 °C) observing five replicates for 24 hr. To determine the percentage of germination on different media, the pollinia after 24 hr of incubation were teased out carefully with a needle to remove the pollinial wall and as many as 1000 pollen grains were studied. The percentage germination varied with the use of different media: 35% in distilled water; 53, 81, 95, 96 and 99% in 5, 10, 15, 20 and 25% sucrose respectively; 85% in Brewbaker and Kwack's medium<sup>6</sup>; 96% in Modified Brewbaker and Kwack's medium (with 20% sucrose and 200 mg/l boron); and 90% in Malik and Chhabra's medium<sup>7</sup>.

## Alexander's staining method:—

Pollen viability was calculated using Alexander's stain<sup>8</sup>, where the fertile pollen were stained red and the sterile ones green. The pollen grains could not be liberated by dissecting the fresh pollinia. Therefore, the pollinia were dipped in double-distilled water for 45 min (time required for initiation of first pollen tube) and then teased out carefully to liberate the pollen grains for staining. The pollen grains (78.9%) stained red, indicating their viability.

Though earlier attempts<sup>1</sup> to culture the pollinia of *C. gigantea* through hanging drop technique did not succeed, it has been successfully carried out here. However, teased out pollen grains from the pollinia of *C. gigantea* cannot be germinated well on any media. A detailed study on the *in vitro* pollinial germination of *C. gigantea* will be published elsewhere.

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## TERATOGENIC EFFECTS OF SODIUM DIETHYLDITHIOCARBAMATE IN THE GARDEN LIZARD, *CALOTES VERSICOLOR*.

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SODIUM diethyldithiocarbamate (DEDIC) and related compounds are of considerable pharmaceutical value. They are used to treat heavy metal poisoning, aversion therapy for alcoholism and as sarcopticide in the treatment of scabies<sup>1,2</sup>. Since our earlier work showed that DEDIC is a potent teratogen in frog embryos<sup>3</sup>, the same was tested on lizard embryos for the degree of its potency.

The eggs were collected from the local garden, cleaned with distilled water and kept on cotton soaked in distilled water. Before experimentation the age of one embryo from a clutch was determined as described by Muthukkaruppan *et al*<sup>4</sup>. The eggs were treated with various concentrations of aqueous sodium diethyldithiocarbamate solution (DEDIC) by keeping them on soaked cotton in petri dishes. Eggs kept on cotton soaked in distilled water were treated as controls. The experiments were conducted at room temperature (26 ± 3°C) and continued until the embryos in the controls hatched out of shell. During this period (40–44 days) cotton was soaked with fresh solution every 72–96 hr. The eggs which showed discharge of fluid from the shell were opened because eggs discharge fluid either when embryos are in hatching stage or are under stress<sup>5</sup>. The embryos were observed under binocular dissecting microscope and photographed.

*Calotes* eggs selected for the experiments were between stage 28 and 30 with 30 to 32 somite pairs. In stage 30 well-marked, swollen limb buds could easily be observed. The period required for complete development and hatching varied between 41 and 44 days. Throughout this period the eggs showed gradual increase in size. In any one clutch, all the eggs hatched within a period of 24 hr from the one that hatched first. All control lizards were normal in appearance and behaviour (table 1).

All experimental egg groups were opened with scissors since hatching failed. Eggs treated with 0.05% and above concentrations showed premature discharge of watery fluid and embryos removed from the eggs were usually found dead or moribund and highly retarded in growth (figure 1). Some embryos died