one set, 2% suspension of this powder in 0·5 N H_2SO_4 was autoclaved at 121° C for 20 min. filtered and the sugars were estimated from the clear filtrate, by phenol sulphuric acid method[4] and copper arsenomolybdate method[5]. In another set, 100 g of the powder was taken in each of the two, 2 litre Erlenmeyer flasks. The ingredients of Waksman No. 77 medium were dissolved in 0·5N H_2SO_4 and added to the flasks to a solvent substrate ratio of 2·5 : 1. Mannitol was eliminated in one of the flasks and both mannitol and yeast extract were eliminated in the other. They were autoclaved at 121° C for 20 min. Later, they were neutralized by adding 25 ml of 5N KOH or NaOH in one and with 5N NH_4OH in the other. Neutralization with NH_4OH added ammonium to the medium and served as nitrogen source to the growing rhizobia.

Fermentation

One ml quantity of actively growing cultures of *Rhizobium* sp. and *A. chroococcum* were inoculated into each of the two flasks and incubated at room temperature (about 30° C) for five days with occasional shaking. Samples were drawn in the initial period and after five days. Ten fold dilutions were prepared and the population of *Rhizobium* sp. and *Azotobacter* were enumerated.

Coffee powder contains mostly cellulose and smaller amounts of oil (caffeol), water, reducing sugar (glucose), dextrin, protein, caffeine, coffearine and tannin. Roasting the seeds at 200–250° C caramellizes the sugar, reduces the moisture and transforms the tannin probably to caffeinic acid and quinic acid[6,7]. Thus the used coffee powder forms a rich source of cellulose.

When coffee powder was hydrolysed with 0·5 N H_2SO_4 at 121° C for 20 min, about 50% of the total sugars was released containing about 28% of reducing sugars. Control flasks where coffee powder was hydrolysed with distilled water released only 1% of the total sugars and contained only 0·6% of reducing sugars. This did not vary much when the time was increased to 30 min and acid, substrate ratio was reducedupto 2·5 : 1.

The population of *Rhizobium* sp. and *A. chroococcum* grown on coffee powder hydrolyzates and enumerated on appropriate media indicated that there was about 4·5 fold increase in the population of *Rhizobium* sp. and about three fold increase in the population of *A. chroococcum*. The population of *Rhizobium* sp. and *A. chroococcum* were 56×10^3 and 78×10^3 in the initial period and were 175×10^8 and 195×10^6 at the end of 120 h respectively. The difference in their growth could be due to the ability of the former to utilise glucose and even pentose whereas the latter can utilise only glucose[8]. The enriched coffee powder waste had about 60% moisture at the end of five days and could conveniently be supplied to farmers without any further treatment.

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A SEARCH FOR SCILLADIENOLIDES IN *SCILLA INDICA* ROXB.

Scilla indica Roxb. is used as a substitute in India for the true or white squill, *Urginea maritima* and the Indian squill, *U. indica* Kuntl.[1]. *S. indica* has also the same therapeutical properties[2]. The commercially available samples of Indian squill are mixtures of *U. indica* and *S. indica*[1,2] and most of the previous phytochemical investigations of Indian squill were based on the two species[3–4]. Rangaswami and Subramaniam[5] isolated a microcrystalline fraction of high biological activity from *S. indica*. But no known scilladienolides were reported by them from this species. Based on the investigation of commercial samples of South Indian squill, Krishna Rao and Vimla Devi[7] reported β-sitosterol from petroleum ether extract of the drug. Krishna Rao and Rangaswami[8] recorded six bufadienolides including scillaren A and proscillaridin A along with a new cardiac glycoside from *S. indica* although their experimental material was the commercial sample of South Indian squill. As the commercial samples are composed of a mixture of these two species, the present investigation was undertaken for qualitative and quantitative analysis of bufadienolides of *S. indica* alone and to compare it with that of *U. indica*[4] in order to ascertain precisely the sources of this compound.

Bulbs of *S. indica* were obtained from the Botanical Survey of India, Pune, and were collected from Bombay and Mahabaleswar. Out of the four populations collected, three were diploid ($2n=30$) and one was tetraploid ($4n=60$). The bulbs were grown in the experimental plot and harvested during active period of growth.

The bulbs were sliced, dried and powdered. The method of extraction of sterol and bufadienolides was the same as of earlier authors[7,8]. The glycoside extracted was examined for the presence of scillaren A and proscillaridin A by thin-layer chromatography using methylene dichloride: methanol: formamide (87 : 12 : 1) as solvent system[9]. The detection was done by spraying with a mixture of 10 ml of aqueous solution of chloramine T and 40 ml of 25% ethanolic solution of trichloroacetic acid[10]. The plates were heated at 110° C for 10 min whereby the glycosides gave yellow fluorescence under long wave UV light. The detection reagent was sensitive for < 5 μg/spot.

The TLC of petroleum-ether extract in solvent system chloroform : benzene (1 : 1) gave negative response to Liebermann–Burchard reagent[10]. β-sitosterol reported to be present[7] was not detected in any of the four populations studied.

The two reference bufadienolides, scillaren A and proscillaridin A could not be detected in any of the four populations. These data are not in conformity with reports of Rangaswami and Krishna Rao[6] and Krishna Rao and Rangaswami[8] who based investigations on commercial samples of South Indian squill. It is likely that the biological potency of *S. indica*[2] is attributable to some other constituents as reported by Rangaswami and Subramaniam[5] and not to scillaren A which is the principal bufadienolide of *U. maritima* (European squill) and *U. indica* (Indian squill).

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EMBRYOLOGICAL STUDIES IN LAMIACEAE

VII. Development of Endosperm in *Craniotome versicolor* Reichb. Iconogr.

THE Lamiaceae are unique in showing characteristic micropylar and chalazal endosperm haustoria. The first authentic work in the family is that of Schnarf[1] who has studied the development of endosperm in several genera. A survey of the literature indicates that very little work has been done so far on development of the endosperm. The investigations on endosperm development in this family include those of Ruttle[2] on *Mentha*, Carlson and Stuart[3] on species of *Salvia*, Ganguly[4] on *Anisomeles* and *Leonurus*, Murthy[5–8] on species of *Ocimum*, *Leucas*, *Anisomeles* and *Orthosiphon*. Junell[9] and Santha[10] have studied the embryology of a large number of representatives of the Lamiaceae. The present study deals with the development of endosperm in *Craniotome versicolor*. The detailed study on the embryology of this taxon will be published elsewhere.

The endosperm formation is of cellular type. The first division of the endosperm primordium is accompanied by the formation of a transverse wall resulting in the formation of a smaller chalazal chamber and a large micropylar chamber (Fig. A). The next division in both the chambers is longitudinal and is followed by the cell plate formation. Thus two tiers of each of the two cells are formed (Fig. B). The cells of the lower tier further undergo vertical division at right angle to the previous one to organise the four celled chalazal haustorium (Figs. D–F, I). The cells of chalazal haustorium generally acquire dense cytoplasm and show hypertrophied nuclei. The chalazal haustorium bends slightly towards funicular vascular supply (Fig. G). It degenerates by the time the proembryo reaches the heart-shaped stage.

The two juxtaposed cells of the micropylar chamber divide transversely forming two superposed tiers for the two cells each (Fig. C). The upper cells undergo vertical division to organise the 4-celled micropylar haustorium (Figs. D–F, H). The cells of the micropylar haustorium elongate irregularly and extend into the broad micropylar cavity. The micropylar haustorium persists upto the late heart-shaped stage of the proembryo (Fig. G).