Grana

ISSN: 0017-3134 (Print) 1651-2049 (Online) Journal homepage: http://www.tandfonline.com/loi/sgra20

Saponins in Pollen

Sangeetaa Wadhawan & C. Kameswara Rao

To cite this article: Sangeetaa Wadhawan & C. Kameswara Rao (1993) Saponins in Pollen, Grana, 32:4-5, 255-259, DOI: 10.1080/00173139309429989

To link to this article: http://dx.doi.org/10.1080/00173139309429989

Published online: 01 Sep 2009.

Grana 32: 255–259, 1993

Saponins in pollen

SANGEETAA WADHAWAN and C. KAMESWARA RAO

Wadhawan, S. & Rao, C. K. 1993. Saponins in pollen. – Grana 32: 255–259. ISSN 0017-3134.

Phosphate buffered saline (PBS) extracts of pollen of 34 out of 95 angiosperm species (in 40 families) tested, lysed human and/or snake head fish (*Channa striatus* Bloch) erythrocytes during assay for lectins in pollen. The bitter taste of the pollen extracts of these 34 species, the formation of a stable foam on shaking and the ability to lyse erythrocytes, suggested the presence of saponins, which have not, so far, been reported from pollen. Thin Layer Chromatography (TLC) and Mass Spectrum (MS) of the erythrocyte-lysing extracts of pollen of the garden gladiolus (*Gladiolus gandavensis* Van Hout.) confirmed that the pollen contained both triterpenoidal and steroidal saponins. The implications for the presence of saponins in pollen inhaled from the atmosphere, in the diagnosis and management of pollen allergy are discussed.

Sangeetaa Wadhawan and C. Kameswara Rao, Department of Botany, Bangalore University, Bangalore 560 056, India.

(Manuscript received 15 June 1992; accepted 9 February 1993)

Saponins are triterpenoidal or steroidal glycosides which occur in various tissues of many plant species, including several food plants such as spinach, beetroot, soybean, tea, etc. (Birk 1969). Saponins are of great interest because the steroidal saponins have hormone-like effects and the triterpenoidal saponins mimic the effects of corticotropin. The steroidal saponins diosgenin, smilagenin, glycyrrhizin, etc., are used as precursors in the synthesis of hormones such as cortisone. The saponins glycyrrhizin and sarasapogenin have therapeutic uses, while some others have industrial applications.

We observed that the phosphate buffered saline (PBS) extracts of pollen of 34 angiosperm species, out of 95 (in 40 families) tested, lysed human and/or snake head fish (*Channa striatus* Bloch) erythrocytes during assay for lectins in pollen. Extracts treated in a boiling water bath for 30 min continued to lyse erythrocytes while dialysed extracts did not, thus ruling out the involvement of a lysing enzyme.

The erythrocyte lysing pollen extracts were bitter to the taste and formed a stable foam on vigorous shaking; these three factors are characteristic of saponins (Birk 1969, Gibbs 1974, Harbone 1984, Dawson et al. 1986). Saponins have not previously been reported from pollen, although steroidal compounds in some pollen (Standifer et al. 1968), and gonodotropin hormone-like steroidal substances in date pollen are known (Hassan & El Waffa 1947, El Ridi 1960, Heftman 1967, Paris & Moyse 1967). Since the presence of saponins in pollen is of interest, we studied the erythrocyte lysing pollen extracts of the garden gladiolus (*Gladiolus gandavensis* van Hout.) in detail, and found evidence for the presence of both triterpenoidal and steroidal saponins.

"This paper is dedicated to the memory of Professor M. Krishna Rao (Department of Botany, Andhra University, Waltair 530 003, India), an exemplery student, teacher and researcher, who passed away prematurely in December 1992."

MATERIAL AND METHODS

1. Pollen material

Mature but unopened flowers of the species listed in Table I were collected from plants growing in and around Bangalore. Flowers of gladiolus were obtained from commercial sources.

2. Extraction of saponins

The anthers were homogenised in a suitable volume of DW, PBS or 80% ethanol using a glass mortar and pestle and the suspension was centrifuged in a microcentrifuge at 8,000 × G for 15 min.

3. Erythrocytes

We have found that snake head fish erythrocytes were four times more sensitive to lysis by saponins than human erythrocytes. Consequently, we have mostly used snake head fish erythrocytes thus enabling us to detect very small quantities of saponins. Typed human erythrocytes of the four blood groups in the ABO system were obtained from the Jayadeva Institute of Cardiology, Bangalore. Erythrocytes of snake head fish were obtained from the Fishery Biology Laboratory, Department of Zoology, Bangalore University.

4. Lysis of erythrocytes

50 µl of the PBS extracts of pollen were mixed with 50 µl of a 2% suspension of human or snake head fish erythrocytes in PBS, in the wells of a glass VDRL plate. Whole pollen scooped out of the anthers were also used. The contents of the wells were mixed well at regular intervals and observations for the lysis of erythrocytes were made at 10, 15, 20, 30, 45, 60 and 120 min (Fig. 1).

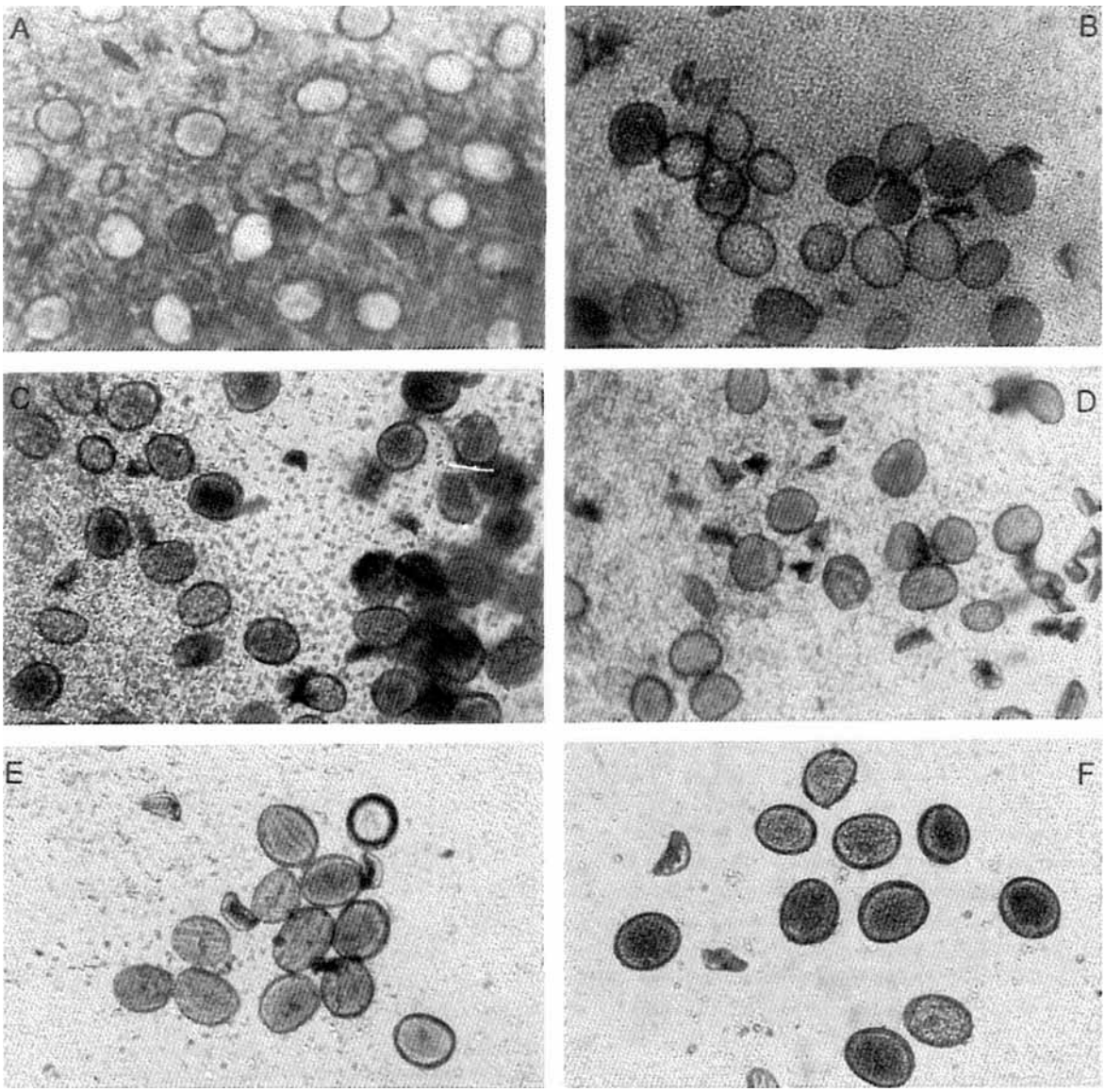

Fig. 1. Lysis of fish erythrocytes by saponins that diffused into the medium from the pollen of gladiolus. The photomicrographs taken at zero (A), 10 (B), 15 (C), 20 (D), 30 (E) and 45 (F) min, show progressive lysis of the erythrocytes. × 225.

5. Thin layer chromatography

Ethanol (80%) extracts of pollen of gladiolus scooped out of the anthers, were used for TLC. These extracts were dried to evaporate alcohol, redissolved in chloroform and subjected to separation on slica gel G medium on glass plates or microscope slides with ethyl acetate and hexane (1:9 v/v) as the solvent (Harborne 1984, Furniss et al. 1989).

The following tests were conducted on TLC plates to detect the presence of saponins in the pollen extracts (Dawson et al. 1986):

a) plates were incubated in a glass chamber saturated with iodine vapour. The saponins were indicated by yellowish brown spots;
b) plates were sprayed with 1% vanillin in ethanol, dried for five min, sprayed with acetic anhydride & conc. sulphuric acid (12:1) and heated at 85–90°C until spots appeared. The saponins were indicated by yellow spots turning grey and then blue-black on a pinkish-grey ground colour (Fig. 2C);
c) plates were sprayed with 1% diamino benzaldehyde in ethanol, dried for five min, sprayed with acetic anhydride & conc. sulphuric acid (12:1) and heated at 85–90°C until spots appeared. The saponins were indicated by rose spots turning yellow;
d) plates were sprayed with 10% antimony trichloride in chloroform and heated at 70–90°C for 40 min. The steroidal saponins were indicated by bright yellow spots on a creamy white ground colour (Fig. 2F);
e) plates were sprayed with 1% vanillin in methanol with 1% conc. sulphuric acid and heated at 90°C for five min. The saponins were indicated by yellow spots turning grey on a creamy white ground colour;
f) plates were dipped in 25% trichloroacetic acid in chloroform and heated at 100°C for 20 min. The saponins were indicated by yellow spots.

Blank TLC plates (Fig. 2A, D) and plates spotted with an extract of pollen of *Eucalyptus tereticornis* which does not contain saponins (Fig. 2B, E) were treated as in a) to f) above, to serve as controls.

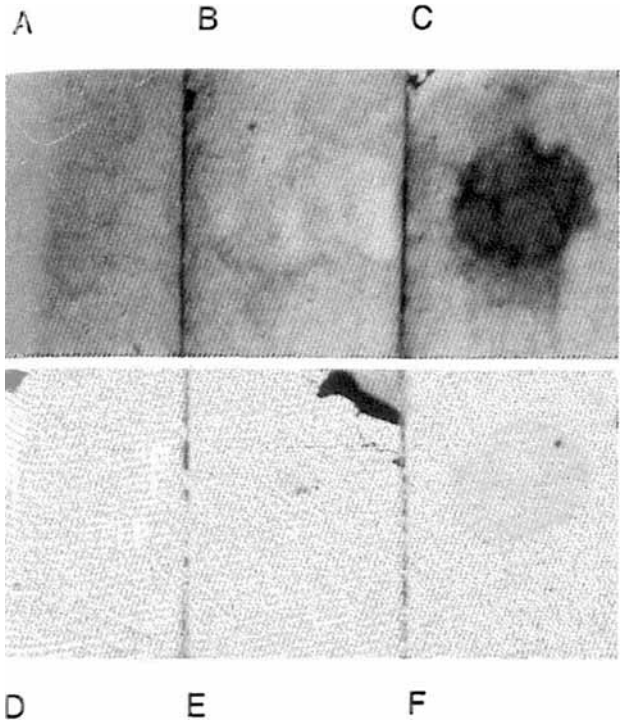

Fig. 2. Detection of saponins in the pollen of gladiolus by TLC. A–C: Treatment with vanillin, acetic anhydride and conc. sulphuric acid. A: blank, B: pollen extract of *Eucalyptus tereticornis* devoid of saponins showed only the residual colour of the extract; C: pollen extract of gladiolus containing saponins. A yellow colour developed on heating indicating the presence of saponins; this turned blue-black on further heating. The ground colour was pinkish grey. D–F: Treatment with antimony trichloride. D: blank, E: pollen extract of *Eucalyptus tereticornis* devoid of saponins showed only the residual colour of the pollen extract; F: pollen extract of gladiolus containing saponins. A bright yellow spot indicated of steroidal sapogenins. The ground colour was creamy white.

6. Mass spectroscopy

Experience has shown that it is advisable to confirm inferences based on TLC and other qualitative data by spectral measurements (Harborne 1984). We, therefore, subjected the eluant (in 80% ethanol) of the spot resolved at Rf 89.3 in TLC to Mass Spectral analysis (Furniss et al. 1989) at the Indian Institute of Science, Bangalore (Fig. 3).

RESULTS AND DISCUSSION

Saponins, evidenced by a bitter taste, foaming on shaking and erythrocyte lysis were detected in the pollen of 34 of the 95 species tested (For list of species see Table I).

When gladiolus pollen, scooped directly out of the anthers, was placed in 50 µl of a 2% suspension of snake head fish erythrocytes in PBS, the saponins diffused out of the pollen and lysed the erythrocytes (Fig. 1). This shows that the saponins actually came from the pollen and not from the anther tissue, where we also found saponins. This has not been reported earlier either. The saponins appear to be localised in the cytoplasm.

Three spots of the extracts of gladiolus pollen with RF × 100 values of 89.3, 16.1 and 6.8 were resolved in TLC. Of these, only the eluant of the spot at 89.3 foamed on shaking and lysed erythrocytes, thus indicating the presence of saponins. The tests done on TLC plates with the extracts of pollen of gladiolus yielded the type of reaction experted of saponins (see Material & Methods). The results of two of these tests are illustrated in Fig. 2.

When subjected to MS analysis, the eluant of the spot at 89.3 showed evidence of both triterpenoidal and steroidal aglycones (sapogenins) (Fig. 3), confirming the presence of saponins in the pollen extracts.

The 34 species delimited here which contain saponins in the pollen are taxonomically diverse. Consequently, it is quite possible that saponins are present in the pollen of a larger number of other species. Considering the known number of angiosperm species, the 95 tested here form too small a sample to allow any generalisations about the distribution of saponins in pollen. However, it is tempting to suggest that families such as Acanthaceae are not likely to contain saponins in their pollen while families like Cucurbitaceae, Malvaceae, Solanaceae, Bignoniaceae and Fabaceae are more likely to show saponins in pollen (Table I).

The physiologial role of saponins in plants is still an unsettled question. The significance of endogenous saponins in pollen cannot be assessed at present because the effects of exogenous phytosterols (Matsubara 1971), saponins (Matsubara 1971) and the detergents Triton X-100, Tween 20 and Tween 60 (Shivanna 1972, Kapoor 1976) on pollen germination and pollen tube growth are inconsistent and contradictory. The hormone-like effects of saponins, particularly steroidal saponins, may have a role in pollen germination and tube growth.

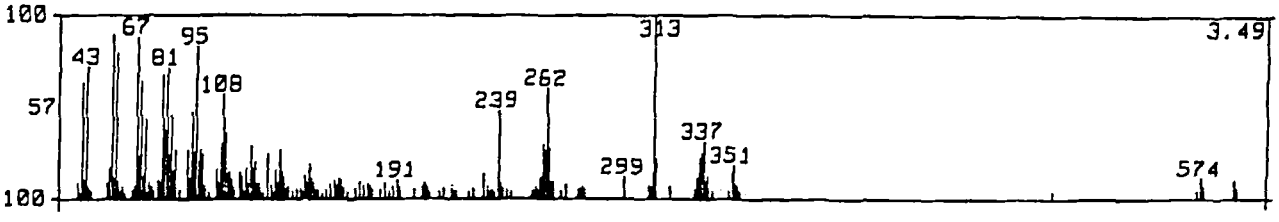

Fig. 3. MS of saponin containing extract of gladiolus pollen (eluant of TLC spot at 89.3) showing triterpenoidal and steroidal aglycones. The intensities of M/Z 239, 262, 313, 337 and 351 indicate steroidal components.

Pollen of *Crotalaria verrucosa, Ricinus communis, Solanum grandiflorum, Zea mays*, etc., are known to be dispersed through the air and are also allergenic (Nair et al. 1986). We found that the pollen of these, and a few other species, contained saponins. The presence of saponins in pollen inhaled from the atmosphere, has the following important implications for the diagnosis and management of allergies:

a) nasal irritation and watering are considered as symptoms of allergic rhinitis caused by pollen proteins. Pollen saponins can be readily released on to the moist nasal membrane and can cause irritation and watering of the nostril, an effect similar to that of soap solution. We personally experienced this with extracts of the pollen of gladiolus. Consequently, all cases of rhinitis need not necessarily be due to allergenic proteins in inhaled pollen. Some of them may be due to the effects of saponins in pollen but have been mistaken for allergic rhinitis;
b) saponins from inhaled pollen can affect the integrity of the nasal membrane, capillaries and erythrocytes. The solubilisation of biological membranes by natural saponins and synthetic detergents is well established (Schnaitman 1971, Haapalla 1973). Using gladiolus pollen, we observed a gelification of the opercular membrane, gill tissue, the muscle layered wall of larger blood vessels and capillaries and intracapillary lysis of erythrocytes in the snake head fish (Kameswara Rao & Sangeetaa, in preparation). When the nasal membrane, capillaries and erythrocytes are affected by the saponins in pollen, the patient's discomfort can increase;
c) When total pollen extracts are used in dermal tests in the diagnosis of allergy, saponins in the pollen can confuse the results because of their membrane solubilising effect on capillaries and erythrocytes. This effect results in an inflammation and redness of the test site which may be confused with the symptoms of allergic response (erythmea and weal);
d) the saponin from the bark of *Quillaja saponaria* acted as an adjuvant and enhanced antigen-specific immune response to an experimental HIV-1 vaccine (Wu et al. 1992). This suggests that saponins in inhaled pollen may also influence the immune reponse to the allergens in pollen.

In view of these considerations we feel that it is important to keep in mind, the possible effects of saponins in pollen, during the diagnosis and mangement of allergy.

LIST OF SPECIES TESTED FOR SAPONINS IN POLLEN

* = species containing saponins in their pollen.

Acanthaceae
Adhatoda zeylanica Medic., (= *A. vasica* Nees); *Barleria cristata* L.; *Peristrophe bicalyculata* (Retz.) Nees; *Thunbergia alata* Boj.; *Thunbergia mysorensis* (Wt.) Anders.

Amaranthaceae
**Aerva lanata* (L.) Juss.

Amaryllidaceae
Pancratium zeylanicum L.; *Zephyranthes citrina* L.

Apocynaceae
Allamanda cathartica L.; **Catharanthus roseus* (L.) Don, (= *Vinca rosea* L.); *Nerium indicum* Mill.; *Plumeria rubra* L. (Red flowers); *Plumeria rubra* L., forma *acutifolia* (Poir.) Wood (White flowers); *Thevetia peruviana* (Pers.) Schum., (= *T. neriifolia* Juss. ex Steud.)

Asclepiadaceae
Asclepias curassavica L.; *Calotropis gigantea* (L.) R. Br.

Asteraceae (Compositae)
**Eclipta alba* (L.) Hassk., (= *E. prostrata* (L.) L.); *Spilanthes calva* DC.; *Tithonia diversifolia* (Hems.) Gray.

Bignoniaceae
**Bignonia magnifica* Bull.; **Haplophragma adenophyllum* (Wall.) P. Dop.; *Millingtonia hortensis* L. f.; **Spathodea campanulata* Beauv.; **Tabebuia argentea* (B. et S.) Britt.

Boraginaceae
Trichodesma indicum (L.) Lehm.

Caesalpiniaceae
Bauhinia purpurea L.; *Bauhinia tomentosa* L.; *Caesalpinia pulcherrima* (L.) Swartz; **Cassia auriculata* L.; *Cassia biflora* L.; *Cassia fistula* L.; *Cassia hirsuta* L.; *Cassia renigera* Wall. ex Benth.; *Cassia roxburghii* DC., (= *C. marginata* Roxb.); *Cassia spectabilis* DC.; **Cassia surattensis* Burm.; *Delonix regia* (Boj. ex Hk. f.) Raf.; *Peltophorum pterocarpum* (DC.) Back. ex Heyne, (= *P. ferrugineum* (Decne.) Benth.); *Pterolobium hexapetalum* (Roth) Sant. et Wagh, (= *P. indicum* A. Rich.); *Saraca asoca* (Roxb.) de Wilde, (= *S. indica* auct., non L.); *Tamarindus indica* L.

Caprifoliaceae
Lonicera leschenaultii Wall.

Caricaeae
Carica papaya L.

Convolvulaceae
Ipomoea hederifolia L., (= *Quamoclit phoenicea* (Roxb.) Choisy); **Ipomoea staphylina* R. et S.

Cucurbitaceae
**Cucumis melo* L.; **Cucurbita maxima* Duch. ex Lam.; **Cucurbita pepo* L.; **Luffa acutangula (L.) Roxb.*

Ehretiaceae
Cordia subcordata Lamk.

Euphorbiaceae
Jatropha glandulifera Roxb.; **Ricinus communis* L.

Fabaceae
Butea monosperma (Lam.) Taub., (= *B. frondosa* Koen. ex Roxb.); **Castanospermum australe* Cunn.; *Crotalaria evolvuloides* Wt. ex Wt. et Arn.; *Crotalaria mysorensis* Roth; **Crotalaria verrucosa* L.; **Erythrina variegata* L., var. *orientalis (L.) Merr.*, (= *E. indica* Lam.); **Sesbania sesban* (L.) Merr., (= *S. aegyptica* Pers.)

Geraniaceae
Pelargonium sp.

Iridaceae
**Gladiolus gandavensis* Van Hout.

Lecythidaceae
Couropita guianensis Aubl.

Linaceae
Linum usitatissimum L.

Lythraceae
Lagerstroemia indica Pers., (= *L. flos-reginae* Retz.)

Magnoliaceae
Michelia champaca L.

Malpighiaceae
Hiptage benghalensis (L.) Kurz, (= *H. madablota* Gaertn.)

Malvaceae
**Hibiscus vitifolius* L.; **Malvaviscus arboreus* Cav., var *penduliflorus* (DC.) Shery; **Sida acuta* Burm.; **Thespesia populnea* (L.) Corr.

Meliaceae
**Azadirachta indica* A. Juss.

Moringaceae
Moringa oleifera Lamk., (= *M. pterygosperma* Gaertn.)

Myrtaceae
Eucalyptus tereticornis Sm.

Nyctaginaceae
**Mirabilis jalapa* L.

Nymphaeaceae
Nymphaea nauchali N. Burm., (= *N. stellata* Willd.)

Papaveraceae
Argemone mexicana L.

Passifloraceae
Passiflora incarnata L.

Plumbaginaceae
Plumbago zeylanica L.

Poaceae
**Zea mays* L.

Punicaceae
Punica granatum L.

Rubiaceae
**Ixora coccinea* L.; *Hamelia patens* Jacq.

Solanaceae
**Datura metel* L., var. *alba*; **Datura metel* L., var. *metel*; **Solanum grandiflorum* Ruiz. & Pav.; **Solanum torvum* Sw.

Sterculiaceae
Firmiana colorata (Roxb.) R. Br., (= *Sterculia colorata* Roxb.); *Waltheria indica* L.

Turneraceae
Turnera ulmifolia L.

Verbenaceae
Clerodendrum inerme (L.) Gaertn.; *Duranta repens* L.; *Gmelina philippensis* Cham., (= *G. hystrix* Schultz. ex Kurz); **Holmskioldia sanguinea* Retz.; *Vitex negundo* L.

Zygophyllaceae
**Tribulus terrestris* L.

ACKNOWLEDGEMENTS

We thank Professor G. S. R. Subba Rao, Indian Institute of Science, Bangalore and Professor R. T. Coutts, Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Canada, for help with MS; Professor J. B. Harborne, School of Plant Sciences, University of Reading, UK, for providing literature; Professor S. Ravichandra Reddy and Dr Narasimha Rao, Department of Zoology, Bangalore University, for fish blood; and the Department of Ecology and Environment, Government of Karnataka, Bangalore, India, for infrastructural support.

REFERENCES

Birk, Y. 1969. Saponins. – In: Toxic constituents of plant food stuffs (ed. I. E. Liener), pp. 169–210. – Acad. Press, New York.
Dawson, R. M. C., Elliott, D. C., Elliott, W. H. & Jones, K. M. 1986. Data for biochemical research, 3rd ed. – Oxford Sci. Publ., Oxford.
El Ridi, M. S. 1960. Gonodotropic hormones in the pollen grains of date plam. – Z. Naturforsch. 15B: 45.
Furniss, B. S., Hannaford, A. J., Smith, P. W. G. & Thatchell, A. R. 1989. Vogel's textbook of practical organic chemistry. 5th ed. – Longman Sci. & Tech., Essex.
Gibbs, R. D. 1974. Chemotaxonomy of Flowering Plants. – McGill-Queen's Univ. Press, Montreal.
Haapalla, E. 1973. The growth of the primary root and root hair of *Sinapis alba* and *Lepidium sativum* in Triton-X-100. – Physiol. Plant. 28: 56–60.
Harborne, J. B. 1984. Phytochemical Methods, 2nd ed. – Chapman & Hall, London.
Hassan, A. & El Waffa, H. M. A. 1947. Oestrogenic substance in the pollen grain of date plam tree. – Nature 159: 409.
Heftman, E. 1967. Steroid hormones in plants. – American Perfum. & Cosmet. 82: 47–49.
Kapoor, A. 1976. Studies on the metabolic regulation of germinating pollen. – M.Sc. (Hons.) thes. Punjab Agricult. Univ., Ludhiana, India.
Matsubara, S. 1971. Effects of steroids on pollen germination. – In: Pollen: Development and physiology (ed. J. Heslop-Harrison), pp. 186–189. – Butterwords, London.
Nair, P K. K., Joshi, A. P. & Gangal, S. V. 1986. Airborne pollen, spores and other plant materials of India. – CSIR, New Delhi.
Paris, R. R. & Moyse, H. 1967. Précis de Matière Médicale. – II, 10. – Masson et Cie, Paris.
Schnaitman, C. A. 1971. Solubilisation of the cytoplasmic membranes of *E. coli* by Triton-X-100. – J. Bacteriol. 108: 545–552.
Shivanna, K. R. 1972. Effect of nonionic surfactants on pollen germination and pollen tube growth. – Curr. Sci. 41: 609–610.
Standifer, L. N., Devys, M. & Barbier, M. 1968. Pollen sterols, a mass spectroscopy survey. – Phytochemistry 7: 1361–1365.
Wu, J. Y., Gardner, B. H., Murphy, C. I., Seals, J. R., Kensil, C. R., Recchia, J., Beltz, G. A., Newman, G. W. & Newman, M. J. 1992. Saponin adjuvant enhancement of antigen-specific immune response to an experimental HIV-1 vaccine. – J. Immun. 148: 1519–1525.