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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.

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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 gonadotropin hormone-like steroidal substances in date pollen are known (Hassan & El Wafia 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.

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 x 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 silica 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 rose spots turning yellow;
c) plates were sprayed with 1% diamino benzaldehyde in ethanol, dried for five min, sprayed with acetic anhydride & 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;
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.
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 1).

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 RFx 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 expected 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 physiological 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.
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 (Schneidman 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 (erythema 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 response 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 management of allergy.

**LIST OF SPECIES TESTED FOR SAPONINS IN POLLEN**

* = species containing saponins in their pollen.

**Acanthaceae**

*Aerua lanata* (L.) Juss.

**Amaranthaceae**

*Aerva lanata* (L.) Nees;

**Apocynaceae**

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

**Asclepiadaceae**

*Aseleia surassavica* L.; *Calotropis gigantea* (L.) R. Br.

**Asteraceae (Compositae)**

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

**Bignoniaceae**


**Boraginacea**

*Trichodesma indicum* (L.) Lehman.

**Caesalpiniaaceae**

*Bauhinia purpurea* L.; *Bauhinia tomentosa* L.; *Caesalpinia pulcherrima* (L.) Swartz; *Cassia auriculata* L.; *Cassia biflora* L.; *Cassia fistula* L.; *Cassia hirsuta* L.; *Cassia reniger* Wall. ex Benth.; *Cassia roxburghii* DC., (= *C. marginata* Roxb.); *Cassia speciabilis* DC.; *Cassia surattensis* Burm.; *Delonix regia* (B. et Hk. f.) Raf.; *Peltophorum pterocarpum* (DC.) Back. ex Heyne, (= *P. ferrugineum* (Decne.) Benth.); *Pierolobium 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.

**Caricaceae**

*Carica papaya* L.

**Convolulaceae**

*Ipomoea hederifolia* (= *Quamoclit phoenicea* (Roxb.) Choisy); *Ipomoea staphylinea* R. et S.

**Cucurbitaceae**

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

**Ehretiaceae**

*Cordia subcordata* Lamk.

**Euphorbiaceae**

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

**Fabaceae**

*Butia monosperma* (Lam.) Taub., (= *B. frondosa* Koen. ex Roxb.); *Castanospermum australis* Cunn.; *Crotalaria evolutoloides* Wt. ex Wt. et Arn.; *Crotalaria myosorhiza* Roth; *Crotalaria verrucosa* L.; *Erythrina variegata* L., var. *orientalis* (L.) Merr., (= *E. indica* Lam.); *Sesbania sesban* (L.) Merr., (= *S. aegyptica* Pers.)
Geraniaceae  

Pelargonium sp.

Iriddiacae  

*Gladiolus gandavensis Van Hout.

Lecythidaceae  

Couroupita guianensis Aubl.

Linaeaceae  

Linum usitatissimum L.

Lythraceae  

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

Magnoliaceae  

Michelia champaca L.

Malpighiaceae  

Hipage benghalensis (L.) Kurz, (= H. madabola Gaertn.)

Malvaceae  


Meliacae  

*Azadirachta indica A. Juss.

Moringaceae  

Moringa oleifera Lamk., (= M. pierygosperna Gaertn.)

Myrtaceae  

Eucalyptus tereticornis Sm.

Nyctaginaceae  

*Mirabilis jalapa L.

Nympheaeaceae  

Nymphea nouchali 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.

Solanaeceae  

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

Sterculiacae  

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  

*Tribrechis terresris L.

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