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Common solvents for making extraction of allergenic proteins from plants' pollens for prick tests and related factors: a technical review

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Abstract

Collecting information on influencing factors in developing consistent and high-quality extracts results in accurate diagnosis and effective treatment of type I allergy (IgE mediated). Furthermore, considering that a large number of allergens are currently in practice, any attempt to develop a more effective procedure for preparing extract may be useful. Nowadays, different saline solvents, temperature, incubation time, and PH are being incorporated for preparing allergen extracts. The objective of the current study was to clear and address the commonest of solvent buffers and allied conditions for making extracts of pollens of grasses, trees, and weeds. The literature review was done in Jan 2016 on PubMed and Google Scholar medical search engines without any time limitation. After reading abstracts of 87 articles, finally 37 relevant papers were selected and their full texts were retrieved. In conclusion, 24 full-text papers were recognized appropriate and chosen. The extracted information for papers has been described fully in the text. On the basis of these data, PBS buffer with PH 7.4, temperature of 4 °C and with overnight incubation time, may be the optimized condition in order to have a proper extract for carrying out skin prick tests.

Keywords: Pollens, Plant Proteins, Skin Test, Allergens, Solvents

1. Introduction

An allergy is an unwanted response of the immune system of the body after exposure to an allergenic material that has entered to the body. Although the manifestation of allergies are not presented in everyone, according to a World Health Organization (WHO) report, sensitization rates of allergies are about 40-50% among school children globally (1). Prediction risk of allergenic properties of a protein is one of the main challenges in molecular allergies (2). Indeed, the immunogenicity of an allergenic protein reflects its potency to develop the IgE antibody in the human body system (3, 4). Some structural characteristics of proteins such as solubility, size, stability, and the compactness of proteins may be relevant to allergenicity power (2). Besides these, the level and route of exposure are important factors in immunogenicity (5). The solubility of an allergen in large amounts upon hydrated condition correlates with its allergenic properties (6). To date, more than 8,000 various allergens have been identified (7). Among them, more than 200 air-borne pollens have been recognized as responsible for respiratory allergies ranging from grasses, trees, and weeds respectively (8-10). The speed of release of allergens in extracts are contributed to some factors

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including location of allergenic proteins in pollens, isoelectric points, solubility, or amino acid composition (11). Also, allergic proteins are located in various parts of pollens including the different layers of the pollen wall (exine, intine, or inner layer) and rough endoplasmic reticulum (11, 12). During pollen's, development, allergenic proteins can be followed through transmission electron microscopy immunocytochemical localization technique (13, 14). An allergenic protein needs rapid elution or solubility as a prerequisite to behave as a major allergen. This also determines which allergenic protein derives in contact with nasal mucosa (15). The comparison of the two realities that some allergenic proteins are released rapidly, and the point that rhinitis symptoms aggravate within 30 seconds after exposure, results in the postulation that freedom of allergens under natural conditions is also rapid (6). A skin prick test is the common and recommended method for detection of allergies to pollens, foods, dust, pet dander or dust mites worldwide. Furthermore, optimal management of allergies requires accurate diagnosis of the cause of the allergy that is mediated with the skin prick test. Various extract solutions from sources of pollens, foods, dust, pet dander or dust mites are manufactured with various buffer solvents by many companies and are on the market. The quality of allergen extracts that are used for skin prick tests (SPT) has attracted many scientists' views in recent decades. It has been shown that many factors can influence the content of the extract and its quality (16). Owing to more information on influencing factors in developing consistent and high-quality extracts will result in accurate diagnosis and effective treatment of type I allergy (17, 18) and moreover regarding a large number of allergens currently used, any attempt in achieving a more effective procedure in preparing extract may be helpful (19). Although reviewing the literature shows that there are different saline solvents, temperature, and PH that has been recruited for preparing allergen extracts, one relevant but poorly understood feature of preparing an extract is the most ideal type of solvent buffer, temperature, and PH that have been applied. The objectives of the current study were to clear the details of biochemical characterizations of solvent buffers and allied conditions for making extracts of pollens of grasses, trees, and weeds. Hence, a review of the literature on pollens' extracts has been done to address commonly used solvent and relevant biochemical factors.

2. Strategy of literature review

The literature assessment was done in Jan 2016 on PubMed and Google Scholar medical search engines without any time limitation, but with language limitation of English. The implemented keywords included: "pollen extract, prick test, solvent, allergenic proteins". Subsequently, after reading 144 titles and abstracts of 87 articles, finally, 37 relevant papers were selected and their full texts were retrieved. In doing so, 24 full-text papers were chosen and recruited in the current review (Figure 1). The inclusion criteria were any published English paper on pollen extracts of plants. The exclusion criteria included any papers on extracts of fungi, food allergens, mites, and animals' allergens.

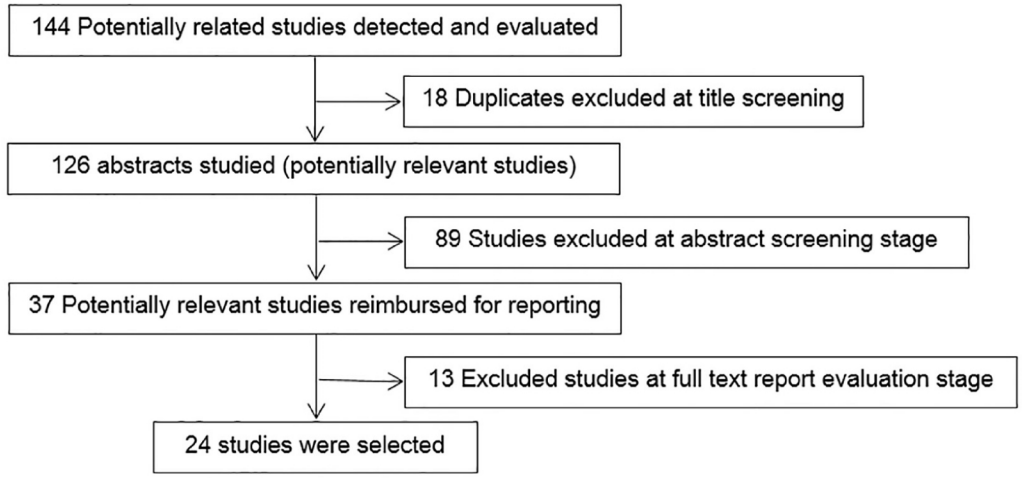

Figure 1. The inclusion and exclusion flow diagram of studied contributions

3. Results

The reported extraction procedures which have been used for preparation of various pollens' extracts comprised an extract of Birch (genus Betula) (5 reports), Timothy (Phleum pratense) (4 reports), Mesquite (Prosopis Juliflora) (4 reports), Ash (Fraxinus excelsior) (3 reports), Cedar (Cupressus sempervirens) and Olive (Olea europaea) (2 reports).

Table 1. The list of reported surveys on used solvents for preparation pollens' extracts before Jan 2016.

Ref. no.	Type of pollen	Type of used solvents	Extraction time (min or h)	Tem (°C)	PH
20	Timothy	0.073 M Tris, 0.024 M barbital, 0.006 M calcium lactate and 0.003 M sodium azide	30 m	25	8.6
12	Olive (Olea europaea)	(50 mM Tris-HCl, 300 mM NaCl, 1 mM EDTA, 1 mM EGTA, 2% Triton X-100, 5 mM ascorbic acid, 100 mM DTT, 2 mM PMSF),	N	N	7.5
6	Birch (Betula verrucosa), Timothy (Phleum pretense)	10 mM potassium phosphate buffer with protease inhibitors, PHEM-Tx buffer with protease inhibitors	2 h	4	7
21	Acacia farnesiana (Vachellia farnesiana)	(PBS) 0.01M	18 h	4	7.4
22	mesquite (Prosopis juliflora)	Ammonium bicarbonate buffer (50mM NH4HCO3, 1mM, PMSF and 2mM EDTA)	4 h	4	8
23	A. retroflexus (redroot pigweed)	PBS, 0.15 M	Overnight	4	7.4
24	Prosopis juliflora (mesquite)	PBS, 0.01 M	18 h	4	7.4
25	Japanese cedar (cryptomeria japonica)	Glycerin 50, NaCl 5%	24 h	5	N
26	White birch (Betula verrucosa)	0.125 M ammonium hydrogen carbonate, 0.015 M sodium azide, 20mM EDTA, 5mM EACA, 1 mM phenylmethylsulfonyl fluoride	20 h	4	7.1
27	Betula verrucosa (white birch)	0.125 M ammonium hydrogen carbonate	20 h	4	7.1
28	Timothy (Phleum pratense)	0.05M ammonium bicarbonate	20 h	4	N
29	Chenopodiaceae (Salsola Kali)	(PBS) 0.01 M	18 h	4	7.4
24	Mesquite (Prosopis juliflora)	(PBS) 0.01 M	18 h	4	7.4
31	Cypress (six genera)	Ammonium bicarbonate	24 h	4	N
30	Ash (Fraxinus excelsior)	Ammonium bicarbonate 4-g/l	24 h	4	N
22	mesquite (Prosopis juliflora)	Ammonium bicarbonate buffer	4 h	4	8
39	Hazel pollen	0.125 M Ammonium hydrogen carbonate, 0.015 M sodium azide, 20mM EDTA, 5mM EACA, 1 mM phenylmethylsulfonyl fluoride (PMSF)	20 h	4	7.1
38	Cedar (Cryptomeria japonica)	NaCl (5%)	24 h	5	N
32	Timothy, olive (O. euro-paea), Ash, (F. excelsior), birch (Betula verrucosa)	PBS	Overnight	4	7.4
33	Ash (Fraxinus excelsior)	PBS	Overnight	4	7.4
34	Amaranthus palmeri	50 mM carbonate buffer	16 h	4	8
35	4 species of Brassica	bmmonium bicarbonate buffer	Overnight	4	7.4
36	maize pollen	PBS	Overnight	N	7.2
37	Shasta Daisy (Chrysanthemum maximum)	PBS	18 h	4	7.4

PH; potential hydrogen, N; not cited in full text, Tem; temperature, h; hours, mM; mili-molar, g/l; gram per liter, °C; degree of Celsius.

Through 24 studied full-text papers (Table 1) (6, 12, 20-39), the following approaches were used:

1) Solvent buffers: Different buffers have been used to prepare pollen extracts. The commonest used buffers included phosphate buffer saline (PBS) 9/24 (37.5%) and ammonium bicarbonate buffer 8/24 (33.3%).
2) Temperatures: The commonest used temperature to prepare pollen extracts were 4 °C with 19 reports (86%), 5 °C - two reports (9.1%) and 25 °C - one paper (4.5%). The minimum and maximum used temperatures were 4 and 25 °C with a mean of 5.04 ±4.46 and median 4.
3) PH: Among the 24 surveys under study, 5 papers did not report using PH, 9 of them had used physiologic PH of 7.4 (47.4%) and three of them had used PH of 8 and 7.1 (5.8%). The minimum and maximum used PH were 7 and 8.6 respectively. The mean of PH was 7.48±0.39 SD and with median 7.4.

Extraction time: The time for preparing pollen extracts varied from 30 min to overnight incubation time. The commonest used incubation times were overnight with 5 cases (20.8%) and 18 hours with 5 cases (20.8%). The lowest reported time used for extraction of pollen proteins was 30 min.

4. Discussion and Conclusions

Among various studied influencing parameters, the used buffer solvent had the widest spectrum of varieties (40). Even there, were varieties among the concentration of specific salts in a certain solvent buffer (41). It seems researchers select their solvent buffer according to their personal experiences. In order to obtain standard extracts, one approach would be the usage of a standard protocol to make the extract. However, this could not be implemented due to a large number of protocols having been used to prepare pollen grain extracts. In biological studies, PBS is used commonly as a buffer solution. PBS is a water-based salt solution, non-toxic, with ion concentration and osmolality similar to the human body. Hence, it can be used as a premium buffer due to similarity with body fluids. This review showed that the most used extraction buffer has been PBS. It seems that relevant factors such as its simple ingredients, non-toxicity, and having similar osmolality with the body has been influencing to select it as an optimum buffer to make an extract. The temperature in which extraction procedure is done, is an effective factor in releasing allergenic proteins from pollens' grains. It has been documented that harsh extraction procedure (boiling pollen grain in the buffer) can discharge non-allergenic proteins, for instance, heat shock protein 70 (41). Incubation in 37 °C can result in releasing of unidentified proteins too. Moreover, it has been shown that thermals induce structural changes in processing proteins (42). Hence, extraction procedure would be done at low temperatures. Accordingly, 79.1% of the reported survey had carried out extraction procedure in 4 °C and only 3 reports had carried out extraction in 35 °C and 5 °C. On the other hand, the temperature and water content of airway mucosa is dynamic between plasma and inspired air, and would be expected to be lower than the core temperature of the body system (43-45). To mimic normal conditions during pollen exposure into the human mucosa and similar releasing allergenic proteins, selection of temperature lower than 37 °C seems a logical option. This reality has been implemented in selection of temperature for extraction, accounting for as much as 79.1% of available literature that have shown to have used 4 °C. It has been demonstrated that fluctuation of PH of a solution can change side chains of the protein and its final shape, and also, small changes in the shape of a protein can lead to a large effect on the manner the protein behaves (46, 47). Proteins may alter their shape in response to changes in parameters such as PH, the polarity of the solvent, temperature and concentration of ions and molecules that can attach to it (48). Going through available literature, confirmed that most reported used PH was physiologic PH of 7.4. This will provide a similar condition in liberating allergenic pollens both on human mucosa and what happens in extraction procedure. Needless to say, with increasing time of extraction, more proteins will have the opportunity to release both in number of proteins and in their concentration into solution. Both overnight incubation and 18 hour incubation times have been used in most procedures and it looks as though there is no remarkable difference between them due to an overnight incubation time taking time as much as 18 h. In short incubation time, there may be a possibility to miss some proteins that need more time for releasing such as the profilin family (a cytoskeletal protein) (48), so, only one paper has reported usage of this timeframe for developing extract. It would appear prudent, on the basis of these data to select extraction buffer of PBS with PH 7.4 and temperature of 4 ^{o}C with overnight incubation time not to miss any proteins from pollen grains, and in order to have a proper extract for carrying out a skin prick test.

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Conflict of Interest:

There is no conflict of interest to be declared.

Authors' contributions:

All authors contributed to this project and article equally. All authors read and approved the final manuscript.

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