CHAPTER 1

Introduction

Allergic rhinitis (AR) is considered as a global health problem and affects over 400 million people all of ages worldwide. In the United States and Europe, more than 40% of population are sensitized with inhalation allergens and the prevalence of AR tends to increase over time, especially in the industrial cities [1-4]. Nowadays, approximately 15-30% of population in the United States are suffering from AR [5]. In Thailand, the prevalence of AR in children was about 13-44% and 20% in adults [6].

AR are not a serious disease but its symptoms mostly troublesome to the patients and usually affects their daily activities, social life, work productivity, school performance, and quality of life [1, 5, 7-10]. Since AR is chronic and persistent AR is very common, long-term use of antiallergic treatment is required, resulting in high losses in both direct cost for drugs and indirect cost for missed school and work days [1]. In Sweden, severe financial losses per year due to rhinitis have been reported [11]. Approximately, $17.5 billion are spent in the US on health-related costs with AR [12].

In the present, there are many available treatment options of AR such as allergen avoidance, pharmacotherapy, immunotherapy, complementary and alternative medicines (CAMs), and surgery. If AR is not properly treated, complications such as nasal polyposis, otitis media with effusion, adenoid hypertrophy, sinusitis, and bronchial hyperresponsiveness or asthma can take place [1, 3-5, 13]. Because of limitations on drugs efficacy, cost, accessibility, and side effects, nowadays, the complementary and alternative medicines (CAMs) are becoming popular. CAMs such as acupuncture, aromatherapy, yoga, homeopathy, nasal irrigation, steam inhalation, and herbal supplement have been described and the patients with AR tend to use these options increasingly. Previous study showed that many herbs such as Chinese herbal medicine, *Petasites hybridus* (butterbur), *Urtica* (stinging nettle), *Sambucus nigra* (elderberry) and

seed) can reduce symptoms of AR [14-17]. Shallot or *Allium ascalonicum* L. is one of Thai herbs that have been recommended to use for AR. According to Thai traditional knowledge, it is suggested that eating ½ to 1 bulbs of shallot per day can reduce AR symptoms [18]. However, the evidence for its efficacy and safety has never been reported.

1.1 Allergic rhinitis (AR)

AR is a chronic illness that involves IgE-mediated immunological response against the inhaled allergens leading to chronic inflammation of nasal mucosa [1, 5]. Various types of allergens such as house dust mites, animal dander, pollen, cockroaches, and molds can trigger different degrees of allergic responses in each individual [2]. Symptoms of AR are characterized by itchy nose, nasal obstruction, sneezing, and rhinorrhea (with clear watery discharge). Moreover, ocular symptoms including watery eyes, itchy eyes, swelling, and eye redness are often found in 50-70% of patients with AR [1, 5, 13].

1.1.1 Diagnosis of AR

The diagnosis of AR is mostly made on the basis of its characteristic symptoms. At least one or more symptoms of itchy nose, nasal obstruction, sneezing, and rhinorrhea must be present. Moreover, the diagnosis could be based on the treatment response to antihistamines or nasal corticosteroids. However, the formal diagnosis should be based on the evidence of sensitization by skin test (presence of wheal and flare as a response to specific allergen exposure) or detection of allergen-specific IgE in serum. Skin test give the result within 15 minutes after testing but the tests for specific IgE in serum take several days to reach the result and less cost effective than skin test. However, the specific IgE tests are valuable for patients who unable to stop antihistamine use [1, 5].

1.1.2 Classification of AR

According to the Allergic Rhinitis with its Impact on Asthma (ARIA) guideline 2008 [1, 3, 4, 19], AR is classified into intermittent and persistent types upon the duration of symptoms.

1. Intermittent allergic rhinitis: patients display the allergic symptoms less than 4 days per week, *or* the symptoms occur continuously for less than 4 weeks

2. Persistent allergic rhinitis: patients have the allergic symptoms more than 4 days per week *and* the symptoms occur continuously for more than 4 weeks

Furthermore, the intermittent and persistent types of AR are subdivided into mild and moderate to severe subtypes based on their severity which affect the patients' sleep or daily activities (Figure 1.1).

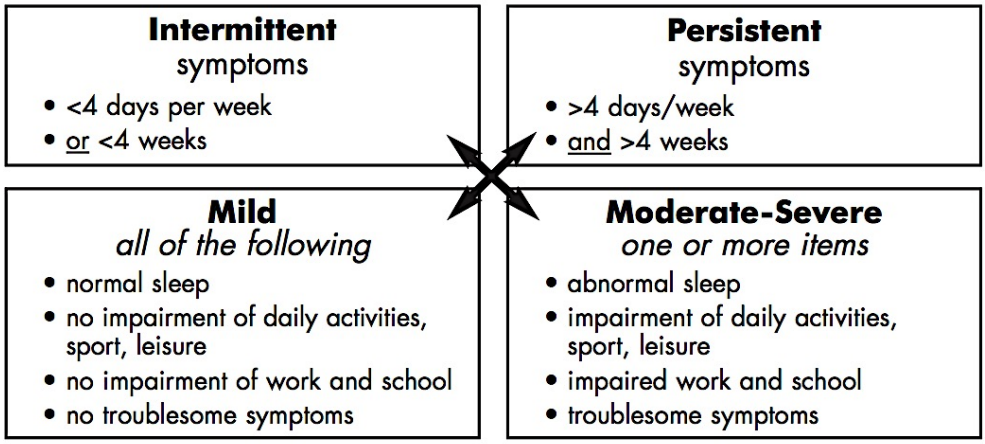

Figure 1.1 Classification of AR according to ARIA guideline [3, 4]

1.1.3 Pathophysiology of AR [1, 5, 19, 20]

Sensitization to allergens

The first sensitization to allergen begins when the antigen presenting cells (APCs) is activated. Dendritic cells in the mucosa surface encounter the allergen and then, present some peptide of allergens in a form of major histocompatibility complex (MHC) class II molecule. The MHC class II and antigen complex combine the T-cell receptors on Naïve $CD4^{+}$T cells, leading to differentiation of Naïve $CD4^{+}$T cells to allergen-specific Th2 cell. The activated Th2 cells subsequently release several cytokines which induce B cells to produce the specific IgE and proliferation of eosinophils, mast cells, and neutrophils. The antigen-specific IgE then binds to high-affinity IgE receptors on mast cells or basophils.

Early and late phase reaction

Subsequently, when AR patients are exposed to that allergen for the second time, allergic reactions will develop in 2 different patterns based on time sequences. Early reaction is the phase that causes sneezing and rhinorrhea. This reaction develops within 30 minutes after allergen exposure and then disappears. The second phase is the late reaction, which is mainly responsible for nasal obstruction. This reaction lasts approximately 6 hours after exposure to allergen.

Early reaction is generally a response of mast cell to the offending allergens, called 'type I hypersensitivity'. The allergen binds to allergen-specific IgE on the surface of the mast cells and circulating basophils and cross-links IgE receptors, leading to activation of mast cells and basophils. The stimulated mast cells induce nasal symptoms for example sneezing, itchy nose by secreting neuroactive and vasoactive mediators such as histamine, prostaglandins, and leukotrienes.

In contrast to the early phase reaction, chemotaxis of eosinophils is the main mechanism for late reaction. This chemotaxis is initiated by the chemical mediators produced in the early phase reaction. Several inflammatory cells, eosinophils, mast cells, and T cells then migrate to nasal mucosa, damage and remodel the normal nasal tissues. These processes mostly result in nasal obstruction which is the main symptom of AR patients.

1.1.4 Management of AR (Figure 1.2)

1. Allergen avoidance

Allergen avoidance is by far the safest and most successful method to cease the AR symptoms. Many AR patients have nasal hyperreactivity to non-specific stimuli such as cigarette smoke, temperature changing, and pollution. Therefore, avoidance of these stimuli is important. However, avoidance of indoor allergens, for example, house dust mites are sometimes difficult and impractical [1, 19].

2. Pharmacotherapy

According to the ARIA 2008, the principle use of pharmacological treatment is a stepwise approach by the duration of AR symptoms and its severity [19].

Antihistamines [1, 5, 19]

Oral antihistamines are the first therapy that use for AR and usually start by the AR patients, because a various of these agents are obtainable over the counter. First-generation antihistamines (e.g. diphenhydramine, chlorpheniramine) own many unwanted side effects, for example, sedation, psychomotor dysfunction, and memory impairment while the second-generation antihistamines (e.g. desloratadine, levocetirizine, loratadine, cetirizine, fexofenadine, rupatadine) less penetrate through blood brain barrier therefore fewer side effects to central nervous system. Additionally, the ARIA 2008 has suggested the use second-generation antihistamines rather than the first-generation. Oral antihistamines are potentially effective for sneezing, rhinorrhea, itchy nose, and eyes symptoms but less effective in nasal obstruction. Moreover, antihistamines are also available as nasal spray. Intranasal antihistamines (e.g. intranasal azelastine, olopatadine) are the topical form of antihistamine which is effective to decrease rhinorrhea, sneezing, and itching but less effective than intranasal corticosteroids and cannot reduce nasal obstruction as well as eyes symptom. However, combination of intranasal antihistamines with oral decongestants can improve nasal airflow in the short term. In addition, these medication offers short duration of action and often cause metallic taste, epistaxis, and mild sedation.

Decongestants

Oral decongestants (e.g. pseudoephedrine) are a potent vasoconstrictor, obviously reduces nasal obstruction via its action on alpha-adrenergic receptors in nasal mucosa but in the short term. Pseudoephedrine usually shrinks the swollen nasal mucosa by reduction of tissue hyperemia and vasoconstriction of mucous membrane. However, side effects from sympathomimetic are frequently reported, for example, hypertension, insomnia, agitation, and tachycardia. However, topical nasal decongestants (e.g. ephedrine, xylometazoline, oxymetazoline) offer faster onset than oral agents but rebound

nasal congestion (rhinitis medicamentosa) is common. There are reports of reduction efficiency as early as 3 days after topical decongestants are used. Therefore, only short-term use is recommended [1, 5].

Anticholinergics

Anticholinergics (e.g. ipratropium) demonstrate satisfactory effect only for rhinorrhea and adverse events such as dry nose, epistaxis, urinary retention, and glaucoma are occasionally observed [1].

Intranasal corticosteroids

Intranasal corticosteroids (e.g. fluticasone, mometasone, triamcinolone, beclametasone) are the most effective drug for AR because of its anti-inflammatory effect on several type of inflammatory cell. Intranasal corticosteroids can inhibit both early and late reactions as well as reduce eosinophilia and IgE production by inhibiting the release of cytokines such as IL-4, IL-5, and IL-13. There have few systemic side effects. Moreover, intranasal corticosteroids are effective in all symptoms of AR including nasal obstruction and eyes symptom. They are superior or equal to the combination of antihistamines and leukotriene receptor antagonists. However, these drugs take several days for reduction of symptoms. The wrongful using leads to treatment failure and adverse events such as epistaxis [1].

Leukotriene receptor antagonists (LTRAs)

The LTRAs (e.g. montelukast, zafirlukast) have been used in asthma but recent evidence confirms their effectiveness in AR patients. The LTRAs are as effective as or softly less than antihistamines. However, its effectiveness is less than that of intranasal corticosteroids. Some studies have shown advantages of combination of LTRAs with antihistamine. In addition, the combination should be inspected for patients who cannot control the symptoms with antihistamines or who do not wish to use an intranasal corticosteroid [1, 5].

Mast-cell stabilizers

Mast-cell stabilizers (e.g. sodium cromoglicate, nedocromil sodium) are generally safe medication with weak effect on nasal symptoms related to AR and the application require several administrations per day [1]. Mast-cell stabilizers act primarily by preventing release of inflammatory mediators from sensitized mast cell through stabilization of mast-cell membranes. They are an alternative to prophylactic treatment of AR and may be preferred for therapy in children. They may also be useful in controlling mild to moderate symptoms [21].

Anti-IgE antibody

Anti-IgE antibody, omalizumab, is an anti-IgE recombinant humanized monoclonal antibody that suppresses inflammatory reactions in blood or nasal mucosa. This anti-IgE antibody interferes the interactions between mast cells, eosinophils and IgE by binding to free IgE, leading to lower amount of serum free IgE. This drug is licensed for patients with severe asthma who associated with AR [1]. However, omalizumab is still expensive and contains adverse effects such as headache and increased opportunity of upper respiratory infection [22].

3. Immunotherapy

Immunotherapy aims to alter the immune system. Subcutaneous immunotherapy is effective in utmost people with AR, with long-lasting reduction of symptoms and necessity of drug requirements. Subcutaneous immunotherapy is given by repeated injections with allergen extracts in increasing concentration until a maintenance dose is reached. It is reserved for people with severe AR whose symptoms are insufficiently controlled with pharmacotherapy or who get side effects from pharmacotherapy. Nowadays, sublingual immunotherapy is considered as method in both adults and children since it is safer than subcutaneous immunotherapy. Many studies suggest that immunological and clinical benefits of sublingual immunotherapy persist after 3 years of continuous use. The risks of immunotherapy are severe systemic reactions or anaphylactic, therefore every patient must be warned and closely monitored after immunotherapy delivery by physicians, especially subcutaneous immunotherapy and first

dose of sublingual immunotherapy [1, 5, 19].

4. Surgery

Surgery is needed very rarely. Usually, surgery is used to treat patients who failure with pharmacotherapy or patients with other complications from AR such as turbinectomy for patients with anatomical deformities or turbinate hypertrophy. Additionally, endoscopic sinus surgery for patients with chronic rhinosinusitis who do not response to pharmacotherapy [1].

5. Complementary and alternative medicines (CAMs)

There are many complementary and alternative medicines that have been used to control AR including Ayurvedic medicine, homeopathy, acupuncture, steam inhalation, aromatherapy, nasal irrigation, and herbal medicine [15, 23]. Many herbs are used to treat respiratory diseases including AR. For example, *P. hybridus* (butterbur), Chinese herbal medicine, *U. dioca* (stinging nettle), *S. nigra* (elderberry) and *N. sativa* (black seed), all are claimed to reduce symptoms of AR [14-17]. In Thailand, *A. ascalonicum* L. or shallot is one of herbal medicine that has been introduced for AR patients. It is suggested that eating ½ to 1 bulb of shallot per day can reduce AR symptoms [18]. However, the evidence for its efficacy and safety has never been reported.

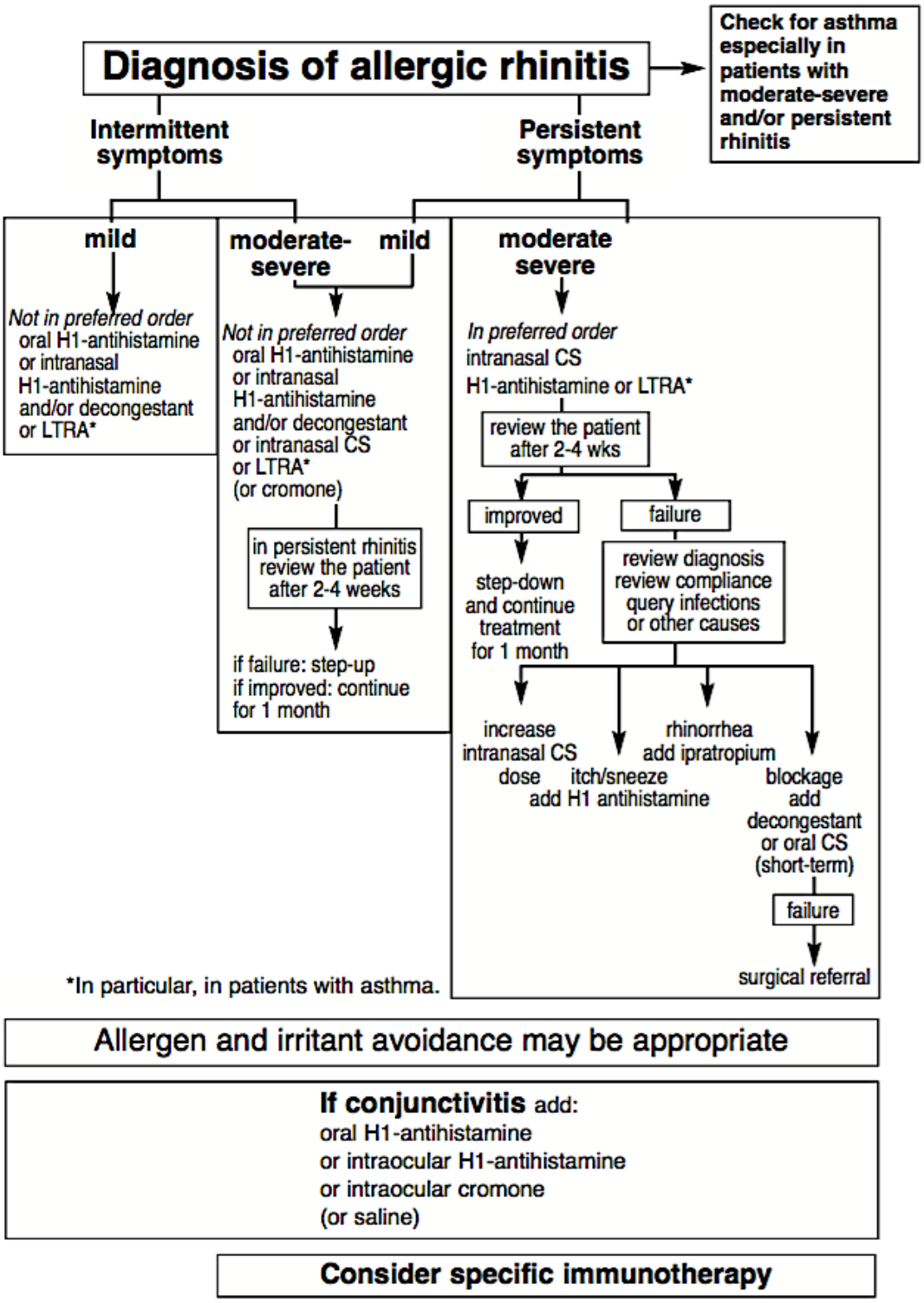

Figure 1.2 Algorithm for AR management according to ARIA guideline [3, 4]

1.1.5 Complications of AR

AR is highly associated with other inflammatory diseases affecting respiratory mucous membranes such as asthma, nasal polyposis, adenoid hypertrophy, rhinosinusitis, otitis media with effusion related to Eustachian tube dysfunction, and allergic conjunctivitis. Allergic conjunctivitis is characterized by itchy eyes, swelling, and discharge. The eyes symptoms are present in 50-70% of people with AR [1, 13, 19].

1.2 Background of medication [10, 21, 24-28]

Cetirizine

Cetirizine, a potent second-generation selective H_1 receptor antagonist, is one of the commonly used drugs for AR and other allergic symptoms including urticaria and chronic idiopathic urticaria.

Pharmacological properties of cetirizine

Cetirizine is white crystalline powder, freely soluble in water with molecular weight of 461.82 and the molecular formula is $C_{21}H_{25}ClN_2O_3$•2HCl (Figure 1.3). In Thailand, each tablet contains 10 mg of cetirizine hydrochloride. Cetirizine is rapidly absorbed following oral administration. Its maximum plasma concentration occurs about 1 hour after and the onset of action is approximately 15 to 30 minutes. Food delay the time to peak plasma concentration but does not decrease the amount of drug absorbed. Cetirizine is bound to plasma proteins in approximately 93%. The metabolism of cetirizine has low first-pass effect. It is metabolized to a limited extent by oxidative O-dealkylation to a metabolite with negligible antihistaminic activity. However, there is no known drug-drug interaction that leads to recommendation of concomitant use avoidance. The mean elimination half-life of cetirizine is 10.5 hours and the apparent total body clearance for cetirizine is approximately 53 mL/min. The excretion of cetirizine is approximately 70% via urine and 10% in feces, respectively in unchanged form.

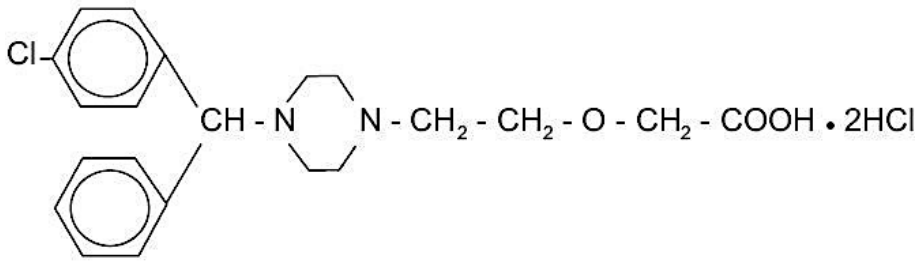

Figure 1.3 Chemical structure of cetirizine

Tolerability of cetirizine

The most common adverse events of cetirizine usually associate with central nervous system, for example, headache and somnolence. Gastrointestinal upset such as abdominal pain, diarrhea, nausea and vomiting may be found in some cases. Other rare adverse events that have been reported include fatigue, dizziness, hypersensitivity reaction, chest pain, rash, confusion, and convulsion. Unavailable about cardiotoxic potential with cetirizine and no harmful effect on pregnant or breastfeeding women.

1.3 Shallot

Shallot or *Allium ascalonicum* L. (*Allium cepa* var. *ascalonicum* Backer); HOM KAENG (หอมแกง); HOM BUA (หอมบัว); HOM LEK (หอมเล็ก) are the plant in Amaryllidaceae family. It is a perennial crop that is grown as an annual for its cluster of small bulbs or cloves. Morphologically, a shallot bulb is very similar to the bulb of the common onion. Moreover, botanically, it is a form of bulb multiplying onion, differentiated by its smaller size. The foliage and inflorescence of shallot is usually smaller than those of the bulb of onion. Shallot is small, layered multipliers with a special taste that falls somewhere between onion and garlic or more highly flavored than the single bulb of onion. Each bulb of shallot produces from four to twelve baby bulbs in a bunch, joined at the base by a membrane. In addition, each bulb is split into two large cloves that may or may not share a common wrapper [29] (Figure 1.4).

In Thailand, shallot is in use as an important ingredient in many dishes in Thai cuisine such as sauces, soup, dressings, compound butters, and to add flavor to simple dishes. Interestingly, shallot is not only as spices for flavoring dishes, but also as medicinal plant to cure many diseases [29]. In traditional use, steam inhalation or soaking

the head with warm water containing freshly crushed bulb of shallot could reduce nasal obstruction from common cold [30]. Moreover, in Thai traditional remedy, it suggested that eating ½ to 1 bulb of shallot per day can help for AR symptoms [18]. However, no scientific study about its efficacy and safety.

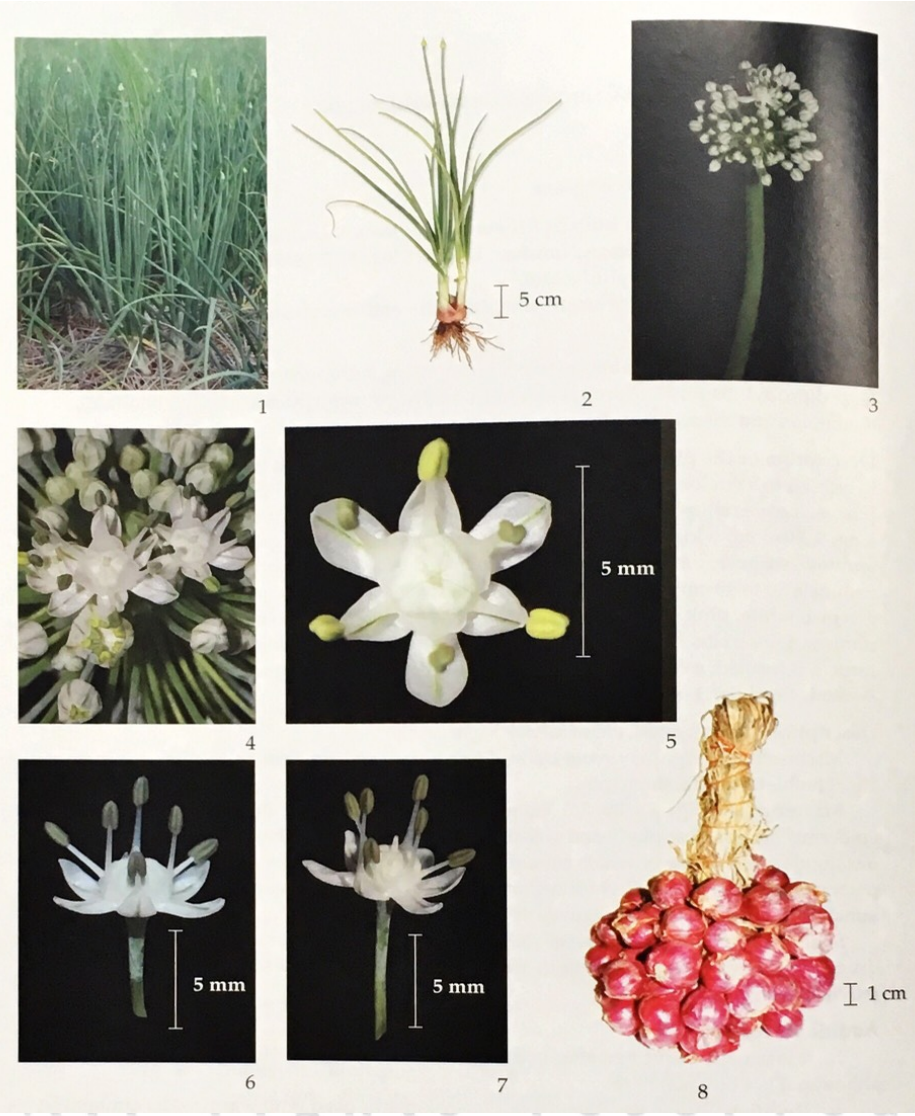

Figure 1.4 Shallot or *A. ascalonicum* L. [30]

1. cultivated plants 2. habit 3. inflorescence 4. flowers (in different stages)

5. flower (top view) 6. and 7. flower (side view) 8. crude drug

1.3.1 Chemical composition

Shallot contain of water approximately 87% v/w [30] with the level of fat and soluble solid, including sugars approximately 16 - 33%. Dried shallot comprises 70 - 85% of carbohydrates which are mainly fructans, glucose, fructose and sucrose. In 100 g of fresh weight of its root, shallot contains water: 79.8 g, calories: 72 g, protein: 2.5 g, fat: 0.1 g, carbohydrate: 16.8 g, fiber: 0.7 g, and ash: 0.9 g. The vitamins and minerals component consist of calcium: 37 mg, phosphorous: 60 mg, Iron: 1.2 mg, sodium: 12 mg, potassium: 334 mg, thiamine: 0.06 mg, riboflavin: 0.02 mg, niacin: 0.2 mg, vitamin C: 8 mg per100 g of fresh weight of root [29]. Shallot contains volatile oil comprising sulfur-containing organic compounds such as alliin (S-allyl cysteine sulfoxide), dipropyl disulfide, dipropyl trisulfide, methyl propyl trisulfide, and diallyl disulfide that give strong odor [30, 31]. Other compound that found in shallot consist of arbutin, calcium oxalate, *A. ascalonicum* lectin, and *A. ascalonicum* lecin AAA [31].

In addition, total phenolic content is approximately 2,528 ± 43 mg/100 g of fresh shallot which gives strong antioxidant property of 70% antioxidant activity [32, 33]. Analysis of shallot extracts has demonstrated the presence of flavone and polyphenolic derivatives such as quercetin, quercetin 4'-glucoside, quercetin 7,4'-diglucoside, quercetin 3,4'-diglucoside, quercetin aglycone, quercetin mono-D-glucose, isorhamnetin 3,4'-diglucoside, and isorhamnetin 4'-glucoside [32, 34-36].

1.3.2 Pharmacological properties of shallot

There are many reports of pharmacological properties of shallot. In Ayurvedic medicine of India, it is used to treat heart diseases, constipation, anti-helminthic, sinusitis, anti-microbial, and wound healing [37, 38]. Moreover, shallot has been reported to have anti-fungal and anti-inflammatory properties in *in vitro* studies [39-41]. Additionally, shallot shows antioxidant effect and free-radicals scavenging ability on cyclosporine nephrotoxicity in rats. It can also reduce the serum level of total cholesterol and low-density lipoprotein (LDL) [33, 42, 43]. Recent *in vivo* and *in vitro* studies have displayed the anti-angiogenesis activity of shallot which is thought to cure or prevent against the angiogenesis-related disorders in the future [44]. In Thai traditional remedy, shallot is commonly used to treat respiratory symptoms including AR but should not be eaten more

than 3 bulbs per day because of its possible side effects such as dizziness and decreased strength of hair roots [18].

1.4 Rhinomanometry (RMM)

RMM is used for evaluation of objectively nasal obstruction. Differentiation methods of RMM include active anterior RMM, passive anterior RMM, active posterior RMM, and postnasal RMM. However, the most common method and also recommended by the International Committee on Standardization of Rhinomanometry is active anterior RMM [45]. RMM is the determination of the difference of transnasal pressure that moves the airflow through nasal cavities. The difference of transnasal pressure is determined between the nasopharynx and the external nares. Airflow in the nose is turbulent airflow that is occurred by the position of the narrowest cross-sectional area of the nose at the nasal valves. Turbulent flow is increased by the complex shape of superior, middle, and inferior turbinates. Therefore, the relationship between pressure and airflow is not linear [46]. Nasal airway resistance (NAR) is calculated according to Ohm's law and following equation [45, 46].

$$R = \Delta P/V$$

When R = Nasal airway resistance ($Pa/cm^3/s$)

ΔP = Transnasal pressure gradient (Pa)

V = Nasal airflow rate (cm^3/s)

The total nasal NAR is calculated by following equation [45]:

$$\text{Total NAR} = \frac{NAR^R \times NAR^L}{NAR^R + NAR^L}$$

When Total NAR = Total nasal airway resistance

NAR^R = Right nasal airway resistance

NAR^L = Left nasal airway resistance

NAR usually presented as a curve plotted diagram (Figure 1.5) on a computer screen of rhinomanometer (Figure 1.6).

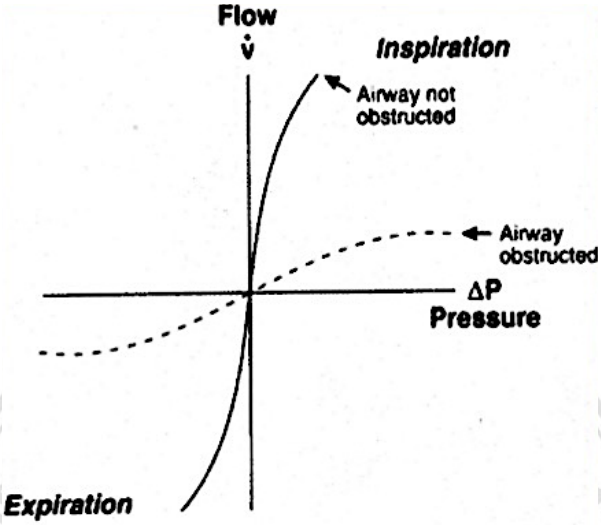

Figure 1.5 Diagrammatic of RMM recording of transnasal pressure against flow during breathing at rest through an unremarkable nose and through an obstructed nose

Figure 1.6 Rhinomanometer

The most appropriate transnasal pressure is 150 pascal (Pa) [45]. However, studies in Thai population recommended at 75 Pa with the total NAR 0.22 ± 0.1 Pa/cm^3/s for Thai people [47]. Additionally, factors such as age, gender, genetic, racial, posture, and exercise may also affect NAR [45, 46].

1.5 Nasal cytology

Nasal cytology was applied for diagnosis of sinonasal disorders, response to treatment, and assessing the characteristics of upper airway inflammation. Many techniques such as nasal wash, nasal brushing, blown secretion, biopsy, and nasal scraping are used for sampling in nasal cytology, but nasal scraping at the inferior turbinate region is easiest to perform, minimally invasive, and painless for the patients. After sampling, processing procedure for nasal cytogram are perform and operate nasal cytogram under microscope with high magnification [48, 49]. The most common cell that usually represent in AR patients by nasal scraping consist of neutrophil, eosinophil, and basophil [50, 51].

1. Neutrophil

Neutrophil has multilobed blue-purplish nuclei with clear or lightly pink-stained and finely granulated cytoplasm [49]. Increasing of number of neutrophils is showed in the nasal secretion in allergic disease. Recruitment of neutrophil occurs in the late phase reaction. Neutrophil can incur airway damage by its lipid mediators such as prostaglandin, thromboxanes, and leukotriene B4 [52].

2. Eosinophil

Eosinophil slightly larger than neutrophils and usually has bilobed blue-staining nucleus. Cytoplasm is filled with distinct large granules and stain reddish-orange [49]. Eosinophil is notable character of AR and is found in nasal secretion and in the airway tissue. Increasing of eosinophil occurs within 30 minutes after expose to allergens challenge. In addition, eosinophil is detected in AR patients only during exposure to allergens. The accumulation of eosinophils and its mediators (e.g. eosinophil cationic protein, major basic protein) have been observed in allergen-induced late phase reaction and result in respiratory epithelium cytotoxicity [52].

3. Basophilic cells (basophil and mast cell)

Basophil contains dominant, large or coarse densely purple-staining basophilic granules. They have bilobed nucleus and smaller than mast cell. Mast cell may take many

shapes. Their nucleus is oval shape with secretory granules that are large and purplish staining [49]. Basophilic cells are also increased in the nose in allergic disease. The normal basophilic cell concentration of the nose is approximately 200 to 400 cells/mm^3 of mucosa. The number of basophilic is significant correlation with level of histamine and nasal eosinophils in AR [52].

4. Lymphocyte

Lymphocyte is the immune cell that is made in the bone marrow. They are divided two main type include B-lymphocytes and T-lymphocytes. B-lymphocytes make antibodies, and T-lymphocytes help control immune responses. Naïve T cells can differentiate into many effector cells such as Th1, Th2, Th9, or Th17 based on status of the cell, cytokines in the microenvironment, and interaction with other cells. However, in allergic disease, Th2 cells produce IL-4, IL-5, IL-9, and IL-13 and result in production of allergen-specific IgE, eosinophilia, the permissiveness of endothelium for the recruitment of inflammatory cells to inflammatory tissues, and the production of mucus [53-56].

5. Macrophage

Macrophage is mononuclear phagocytes or differentiated monocytes that have larger cells and more intracellular organelles. They circulate in the bloodstream and live in different tissues that have special names such as Kupffer cells in the liver, microglial cells in the brain, and osteoclasts in the bone. In addition, they play role of phagocytosis and alarm other cells to the infection. Macrophage activity can be enhanced by the cytokines secreted by activated T-helper cells and by other inflammatory factors in the inflammatory response. Activated macrophages are more powerful for eliminating pathogens. They exhibit greater phagocytic activity, increased secretion of inflammatory factors, and higher expression levels of MHC class II molecules, which can present antigens to T-helper cells. Thus, macrophages and T-helper cells mutually facilitate activating each other. Macrophages not only eliminate pathogens directly but also act as antigen-presenting cells to mount a more effective defense [56, 57].

1.6 Objectives of the study

1. To determine the efficacy and safety of oral supplement of *A. ascalonicum* L. in patients with persistent AR

2. To compare the efficacy and safety of oral supplement of *A. ascalonicum* L. to placebo in patients with persistent AR

3. To observe the possible adverse effects of oral supplement of *A. ascalonicum* L.