Herbs for Voice Database: developing a rational approach to the study of herbal remedies used in voice care

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Abstract

Herbs have been used for voice care since ancient times and many herbal remedies are still in use in every geographical areas and cultures, both as traditional medicine and as sources of botanicals used in commercial products. Many of these plants are used as extracts and other phytopreparates, and a full phytochemical analysis is sometimes incomplete or lacking. The mechanisms of action of these botanicals include anti-bacterial, anti-inflammatory, mucolytic and other general activities; nevertheless, mechanisms that could be specifically referred to voice are often unknown, as well as the corresponding molecular targets and therefore a rational approach in the use of these remedies is hard to be applied by phoniatricians. To address this problem, we collected information on plants used for voice care from several different geographical areas, using both literature data and a pool of contributors from an international network of artistic phoniatrics and vocologists. The plants have been organized in a database (Herbs for Voice Database) and classified according to the natural compounds contained in them, their molecular targets and the pathologies they are recommended for. This first database contains 44 plants, 101 phytocompounds and 32 recognized molecular targets. The distribution of herbs and phytocompounds according to the botanical families, their known biological activity, traditional uses and molecular targets were analyzed. In particular, data analysis shows that the somatosensory and pain receptor Transient Receptor Potential Ankyrin 1 (TRPA1) ion channel is targeted by a large number of different phytochemicals contained in the herbs for voice, and could therefore be involved in a mechanism of action common to many plants.

Keywords

voice; botanicals; database; dysphonia; phytotherapy; TRPA1 ion channel.

Abbreviations

SO	Sisymbrium officinale (L.) Scop.
NO	Nitric oxide
PGs	Prostaglandine

IL	Interleukins
NF-kb	Nuclear factor k-light-chain-enhancer of activated B cells
LOX	Lipoxygenase Enzyme
COX	Cyclo-Oxygenase Enzyme
GABA	γ Aminobutirric acid
5-HT	5-Hydroxytryptamine
TRPA1	Transient Receptor Potential Ankirin 1 ion channel
TRPM8	Transient Receptor Potential Melastatin type 8 ion channel
CNS	Central Nervous System

1. Introduction

Dysphonia is a common symptom that affects nearly one-third of the population at some point in life but has a prevalence among people with demanding vocal loads [1], among which teachers, lawyers, singers, actors, politicians, and many more. [2]. Most vocal disorders do not deserve surgical intervention [3-5], but may solved by many different treatments, often comprising the use of phytotherapy.

Voice was a fundamental tool also in ancient societies, and herbal medicine is the oldest form of healthcare to protect voice and it brings together the practices of ancient physicians and the traditional knowledge of many generations in selecting herbal formulation for the treatment of dysphonia. Among those remedies *Sisymbrium officinale* (L.) Scop. is one of the oldest and most frequently used plants in herbal formulations for voice diseases in Europe and many other countries. *Sisymbrium officinale* (SO), known in English as *hedge mustard*, is an annual wild plant belonging to the Brassicaceae family, native in the Mediterranean area. The traditional name "erysimum" comes from the ancient Greeks Ἐρύσιμον, Ἐρύσ = I save and σιμον = sing. As it can revive voice, it is known as "the singer's plant". Many popular names given in various languages reflect the widespread traditional knowledge about protective activity on voice and vocal tract of this plant.

SO aerial parts are traditionally used as a remedy for airway ailments such as loss of voice, laryngitis, pharyngitis, coughs and asthma. An infusion of the whole plant has been used for all throat diseases [4]. The pharmacological activity of SO shows anti-inflammatory, analgesic, antitussive, myorelaxant [5] and broad spectrum antimicrobial [6] activity and also anti-mutagenic properties [7]. Its effect on alleviating vocal tract disability in a cohort of 104 patients showing various degree of vocal tract discomfort has been recently reported [8].

Recently some of us [9] showed that isopropylisothiocyanate and 2-butylisothiocyanate, the two main isothiocyanates from SO, are strong agonists in vitro of the TRPA1 (Transient Receptor Potential Ankyrin 1) somatosensory receptor, an ion channel involved in the mediation of inflammatory and neurogenic pain [10, 11, 12] suggesting that TRPA1 can be one of the molecular mediator of the therapeutic effects for SO.

Beside SO, many other plants and herbal extracts are commonly used for "over-the-counter" voice care and there is a growing interest for this "symptomatic" approach in clinical practice by phoniatricists and vocologists. Nevertheless, there is a lack of comparative studies on this subject: how many plants are known and employed for voice care all over the world? Which active principles do they contain? Which mechanisms or molecular target related to voice do they have in common? Is there a rational behind their modality of use, the therapeutic indications and the combination of two or more active compounds in a phytopreparation?

Aim of this work is to propose a rational approach to answer these questions, following the same approach already used for SO. To do that, we built a database that we called "Herbs for Voice Database", containing the plants used for voice therapy all over the world. It is based on the collection, classification and comparative analysis of existing data, with the aim to investigate the putative connection between plant, phytocompounds contained therein, their molecular targets and biological activity and to obtain useful information on the molecular mechanisms operating in voice protection by natural compounds from plants.

2. Materials and Methods

2.1 Choice of contributors

A first list of plants used for voice has been collected by one of us (OC) with the contribution of eight phoniatricians experts in the pathologies of artistic voice from different countries (Belgium, Canada, Italy, Poland, Portugal, Russia, Turkey, US), in order to consider a large geographical representation. The contributors (name, affiliation, country) are listed in the database. They provided information about herbal remedies they prescribe or that are traditional in their countries. This has allowed us to include herbs not only for the traditional use but mainly selected for their performances on voice by phoniatricians. All contributors were asked for permission to be cited in the database.

2.2 Literature search

The search for relevant information was performed on the following databases: PubMed, Scopus, Google Scholar, ScienceDirect and European Medicines Agency's (EMA) website and on specialized publications [13].

We used the botanical name of medicinal plants alone and in combination with the following keywords: voice, hoarseness, aphonia, throat, larynx, pharynx, vocal, phonation, cough, respiratory, anti-inflammatory, antinociceptive, analgesic, antibacterial, TRPA1, antioxidant, antitussive.

Keywords have been chosen in order to provide an overall pharmacological assessment of the plants recommended by the contributors and to evaluate the coherence of the use for voice of these herbal remedies.

2.3 Data elaboration and repository

Data have been elaborated with Microsoft Excel Office (2016). The complete file is a matrix of 192 rows, 16 columns and contains 160 bibliographic references. The file has been uploaded in a Dataverse, file name "Herbs_for_Voice_database_public_version_20190503" and published on the Dataverse project (Harvard University). Date of deposition: May 2nd, 2019; accession number: (note for the referees: the data are available to referees as supplementary material; after acceptance, we will add the accession number and the Dataverse will be made available to public.)

3. Results

3.1 Data base building

With the obtained information, we organized a spreadsheet with the following entries: scientific plant name; botanical family name; common name(s) in English; common name in other languages, when available; preparation/parts of the plant that have been studied; isolated active compounds; biological activities of the compounds; molecular targets; effective doses; clinical and traditional uses; toxicity; interactions with other drugs; side effects; contraindications; contributors; bibliographic references.

Information about plants (species name, botanical families and synonyms, when available) were checked for their correct attribution. Some plants were referred by the contributors with the common name instead of the scientific one, therefore the correct attribution of botanical species was evaluated and verified one by one, wherever possible.

For each plant we made a survey on phytochemical analysis in order to find which active substances are contained and which biological targets could be involved, in relation with voice. Since it is known that TRPA1 active substances are involved in nociception and inflammatory pain and that SO contain strong agonists of this ion channel, in our search we highlighted if other herbs in our database contain TRPA1 agonists.

The database reports the main clinical indications and traditional uses which could be related both to voice and to respiratory symptoms like dyspnea, irritation, cold, asthma and similar terms. Other biological effects which are not directly related to voice, such as “antioxidant”, were listed only when cited by many sources or when they are reported to be the primary effect of that plant or compound.

Other information related to medicinal and traditional uses were also collected when available. Among them: the kind of preparations and administrations (e.g. infusions, essential oils etc); the parts of the plant used (leaves, seeds, roots etc); the effective doses; toxicity; side effects; contraindications and interaction with other drugs.

3.2 Analysis of data

A total of 44 plants, 1 fungus and 1 lichen were listed. Among the plants, 40 species were recognized belonging to 23 families. In five case (Drosera spp., Mentha spp., Eucalyptus spp., Rosa spp. and Citrus spp.) we considered the whole genus, because the species could not be identified.

Among the identified plants, some are widely diffused and commonly used, like tea (*Camellia sinensis* (L.) Kuntze), ginger (*Zingiber officinale* Roscoe) and peppermint (*Mentha ×piperita* L.); others are less known and have limited geographical spread, like bitter kola (*Garcinia kola* Heckel) and oroxylum (*Oroxylum indicum* (L.) Kurz). Some plants in the Database are mostly used as food plants, like okra (*Abelmoschus esculentus* (L.) Moench). To our knowledge, only *Sisymbrium officinale* (the “singers’ plant”) is known almost exclusively for its specific activity on voice.

3.2.2 Herbs for Voice vs botanical families

We compared the number of the species belonging to each family, expressed as a percentage, in Herbs for Voice database and in the entire plant domain in the world [14] (Figure 1).

Figure 1. Number of the species belonging to each family in Herbs for Voice database (dark grey) and in the entire plant domain (light grey), taken from literature. Numbers are reported as percentage. Families are listed in alphabetical order.

In Herbs for Voice database the bar graph shows the prevalence of the family *Lamiaceae* (12,5 %), while species owing to this family represent about 8% of all plants. Also *Malvaceae*, *Ranunculaceae* and *Rhamnaceae* are represented in a higher proportion in the database than they are in general. Instead the *Asteraceae*, which is the largest family in the world, accounting around 30% of the total species, reaches no more than 10% of the species in our database.

3.2.3 Herbs for Voice vs biological activity

We analyzed the biological activities associated to the herbs in the Herbs for Voice database.

We found eighteen reported biological activities: anti-inflammatory (41 plants), analgesic (37 plants), antipyretic (2 plants), anti-oedematous (2 plants), anaesthetic (2 plants), antimicrobial (20 plants), antibacterial (25 plants), antifungal (8 plants), antiviral (6 plants), antioxidant (26 plants), antitussive (8 plants), expectorant (7 plants), spasmolytic and myorelaxant (8 plants), secretolytic (3 plants) , immunomodulatory (8 plants), immunostimulant (8 plants), anti-hyaluronidase (1 plant) and tissue and cellular protective (2 plants).

The overall distribution is shown in Figure 2, panel a.

Some activities were merged according to their similarities. Anti-inflammatory activity was merged with anti-oedematous and antipyretic. Antimicrobial was merged with antibacterial, antifungal and antiviral. Antitussive incorporated expectorant, myorelaxant and spasmolytic, anaesthetic and secretolytic. Immunomodulatory and immunostimulant were merged as immunomodulatory. “Others” includes anti-hyaluronidase and tissue and cellular protective activities.

This pooling allowed to highlight that anti-inflammatory, analgesic and antimicrobial are the most important biological activities associated to the Herbs for Voice database. These three activities are correlated with the most common pathologies of voice and upper airways, as cold, hoarseness and laryngitis.

Data were further refined by excluding antioxidant activity, which is not specifically related to voice, as well as “others”. After this filter, the anti-inflammatory, analgesic and antimicrobial resulted to be the most important biological activities in the Herbs for Voice database, accounting for 27, 25 and 25% of the total activity respectively.

The final result is shown in Figure 2, panel b.

Figure 2. Biological activities associated to the herbs in the Herbs for Voice database; data are expressed as percentage. (**a**) all the activities found in the literature; **(b)** main biological activities after merging similar results.

The sum of anti-inflammatory, analgesic and antimicrobial activities account for 77% of the total, followed by antitussive (12%) and immunomodulating (11%) activities.

3.2.4 Herbs for Voice vs associated symptoms and diseases

The analysis of literature renders seventeen symptoms or diseases, for which the plants recorded in the Herbs for Voice database are used: cough, sore throat, pain, irritation, inflammation, improving singing voice, hoarseness, pharyngitis, laryngitis, infection, fever, constipation, cold, bronchitis, mucous protection, dyspnea and asthma (Figure 3, panel a)

As shown in Figure **3** the more common indications are for cough, bronchitis and cold.

Data were then merged into four main categories:

- irritation symptoms, that include cough, sore throat, pain, irritation and inflammation;
- infection effects, that include pharyngitis, laryngitis, infection, fever, constipation, cold and bronchitis;
- respiratory impairments, that include asthma, dyspnea and mucous protection;
- voice quality alteration, e.g. hoarseness and improvement of singing voice.

The result is shown in Figure 3, panel b.

Figure 3. symptoms or diseases for which the plants recorded in the Herbs for Voice database are used. (a) number of plants in the database used for symptoms and diseases described in the literature; symptoms owing to similar categories are shown in different shades of grey. (b) symptoms after merging in four main categories; data are expressed as percentage.

This graph indicates that plants for voice are mostly used as remedy for irritation symptoms (40%) and infection effects (33%), followed by respiratory impairments (19%) and voice quality alteration (8%).

3.2.5 Herbs for Voice vs biological targets

Finally, we analyzed the known biological targets associated with the phytocompounds in the plants listed in the Herbs for Voice Database. We found seventeen different targets: peripheral nervous system; central nervous system; microbial membrane; NO (Nitric Oxide); PGs (prostaglandins); IL (interleukins); macrophages; NF-kb (Nuclear factor k-light-chain-enhancer of activated B cells); LOX (Lipoxygenase Enzyme); COX (Cyclo-Oxygenase Enzyme;) GABA (γ Aminobutyric acid) receptors; dopaminergic receptors; opioid receptors; adrenoreceptors; 5-HT (5-Hydroxytryptamine) receptors; TRPA1 and TRPM8 ((Transient Receptor Potential Melastatin type 8) ion channels. In some cases, they are complex systems as CNS, or microbial membranes. In other cases, the biological targets have been identified at molecular level as for the receptors of GABA or opioids, or the TRPA1 ion channel.

The following diagram (Figure 4) shows the molecular targets and their relative frequency of citation in the database.

Figure 4. The biological targets associated with the phytocompounds of the plants in Herbs for Voice database.

Within the analyzed data, TRPA1 is by far the most represented biological target. Agonists of this ion channel have been identified in 27 over 44 plants, corresponding to 61,3% of the plants in the Herbs for Voice database.

4. Discussion

Despite its importance in human development and communication, voice is often neglected in pharmacological research. Voice care is a multifaceted problem since the phonatory apparatus includes different organs and tissues and is subject to many possible attacks from external agent or undergo malfunctioning due to physical, functional and/or psychological impairment. Beside conventional pharmacological or surgical treatment, herbal remedies are an interesting alternative especially in case of minor pathologies and symptoms. According to the World Health Organization (WHO) about 80% population of most developing countries still rely on traditional herbal medicines for their primary health care needs [15]. Also in western countries, the consumers' demand for herbal remedies is increasing globally [16]. The need of a practical, comprehensive and widely accepted framework to international traditional herbal medicine research has been recently proposed by the WHO [17].

As for many other pathologies, many reports are available about the use of herbal remedies for voice care, but few studies have been made with appropriate and standardized research protocols and therefore the interpretation of outcomes can be doubtful.

The creation of Herbs for Voice database, collecting systematic information on herbal remedies for voice care, is a first step to approach this matter in a rational way.

A few observations emerge from the analysis of preliminary data.

Some herbs are largely employed in traditional medicine as well as in commercial phytopreparates. Beside SO, also ginger is largely used by singers for its known antimicrobial effect. Over and above its effectiveness, ginger is easy to find and has a pleasant taste and flavor, which make it a very popular remedy. These features are also shared by many other aromatic plants such as mint, oregano, sage, cinnamon, eucalyptus and thymus, which are frequently employed in preparations for oral hygiene and mild treatment of the upper respiratory trait.

Some plants in the database are known for specific activities; for instance, *Drosera* is generally employed for cough, *Echinacea* for immunostimulation, *Malva sylvestris* and *Calendula officinalis* are used to soothe irritations as well as okra.

Botanical analysis shows that the family of Lamiacee is the most represented in the database, and this is consistent with the fact that this family includes several aromatic and medicinal plants as those listed above. Moreover, the flavor of these plants is often characterized by somatosensory properties which are mediated by receptors of the TRP ion channel family as TRPA1 (pungency) and TRPM8 (cooling effect). Interestingly, "freshness", is also a desirable feature in products used for inflammation of the oral cavity, and possibly it contributes to reduce the perceived sensation of vocal discomfort.

The reduction of inflammation and the consequent irritation is one of the main target of herbal remedies used for voice. Laryngeal somatosensory feedback plays an important role in regulation of normal upper airway functions, like voice production, and altered sensory feedback from the larynx is known to be involved in a variety of pathological reflex responses including dysphonia. Laryngeal sensory input alterations occur frequently and may be induced by laryngeal inflammation due to voice abuse, upper respiratory infection and irritation, vocal fold trauma and others [19]. Accordingly, the plants in Herbs for Voice Database are mostly used as remedies for irritation symptoms and infection effects and show mainly anti-inflammatory, analgesic and antimicrobial activities. Antitussive activity is described in a few cases, maybe because the communicative value of voice is less hampered by cough, than by infection or inflammation.

Herbs having broader effects on immunological system, as *Echinacea purpurea* and *Oroxylum indicum* are represented in the database in a smaller number (11%). It's interesting to note that in Western Medicine immunology started with Jenner in 1796 and it's a relatively recent acquisition, whereas in traditional Medicines, like Chinese, the existence of a defensive factor - defensive *qi* - was known as well as herbs apt to enhance it. The enlargement of the Database with information coming from Traditional Chinese Medicine will therefore add interesting information on this aspect.

The search for putative common mechanisms operating in plants used for voice care is one of the aims of this study. Medicinal herbs are selected by populations according to their uses derived by trial and error procedures, and are chosen among those offered by local biodiversity. Therefore, to look for common pathways within this plant database could be challenging.

Anyway, the analysis of available data seems to support the hypothesis that some common mechanism can be found. In particular, we found that the plants in the database contain a very large number of natural agonists of the TRPA1 ion channel, a well-known mediator of inflammatory and neurogenic pain. This finding reinforces the hypothesis that this multi-purpose molecular sensor could be involved in some relevant mechanisms of action of these traditional remedies, included that of *Sisymbrium officinale* that has been previously demonstrated *in vitro*.

Figure 5 shows the distribution of the molecular targets in the subfamilies of plants of the database which are associated to analgesic (panel a) and anti-inflammatory (panel b) activity.

Figure 5. Distribution of the biological targets associated with the plants in Herbs for Voice database. (a) targets for plants associated with analgesic activity. (b) targets for plants associated with anti-inflammatory activity.

TRPA1 is the main target (around 50%) of the plants with analgesic activity included in the database, and its percentage is much greater than that of the opioids and GABA receptors, that are typical analgesic targets (both around 20%). TRPA1, followed by NF-kB and COX, is also the most reported target of the plants for voice that possess anti-inflammatory activity.

TRPA1 channel has been identified in many cell types in the airways, principally in airway sensory neurons (spinal dorsal root ganglia, nasal trigeminal and vagal neurons), but also in lung fibroblasts, airway smooth muscle cells, bronchial and alveolar epithelium cells [11, 20]. TRPA1 channel is considered an important part of the lungs defense system and immunohistochemical studies have identified TRPA1 also in oropharynx and larynx [21]. The vagal and trigeminal ganglia innervating airways show appreciable TRPA1 expression [22]. In particular, in the superior laryngeal nerves (SLNs), the main sensory nerves of the larynx, the subpopulation of capsaicin-sensitive afferents are sensitive to various noxious stimuli via activation of TRPA1 channels [23]. It is known that TRPA1 stimulation produce airway neurogenic inflammation and can modulate airway inflammatory response [21], furthermore TRPA1 activation has been linked to trigger bronchoconstriction, cough and airways irritation.

At this point in our database results, TRPA1 is the most represented biological target and some agonists of this ion channel have been identified in 67,5% of all the plants in the database. Besides, it has been recently demonstrated the isolated isothiocyanates from *S. officinale*, the singer's plant, activate and subsequently desensitize the TRPA1 ion channel in vitro [9]. This finding reinforces the hypothesis that this molecular sensor could be involved in some relevant mechanism of action on voice of these traditional remedies, included that of SO.

The ion channel TRPM8 is also a well-represented target in these plants, found in around 30% of cases. TRPM8 is the other thermosensor –beside TRPA1- involved in the perception of cold in humans [24]. This channel is reported to be expressed in lingual nerve fibers of the tongue, neuron trigeminal ganglia, dorsal root ganglia and other tissues [24, 25]. As for TRPA1, TRM8 activation and desensitization are controlled by multiple intracellular signaling pathways. Plants containing cooling principles such as menthol, eucalyptol or eugenol, active both on TRPM8 and TRPA1, are quite commonly used for improving voice and respiratory system performance. The role of both the cold ion channels TRPA1 and TRPM8 in voice protection mechanisms at molecular level could be an interesting point for further research.

In conclusion, the Herbs for Voice Database represents a useful initial step to gain information on the role of botanicals for voice care. A desirable outcome would be the enlargement of the database, especially in the underrepresented areas as Asia, Africa and Australia, that could be obtained with the approach of participatory science by contributors from the scientific community. Many intriguing aspects related to historical, environmental, cultural studies about voice use and care could also benefit by such data collection. Besides, this approach may help physicians to address their patients with voice problems travelling all over the world to find same or similar active principles in different plants in different countries.

The analysis of data will help to formulate hypothesis about the mechanism of action of these herbal remedies, compensating actual lack of scientific bases and explaining physiological effects. Finally, this approach can be useful to suggest more evidence-based treatments in this field and can give useful hints on a more rational use of such phytocompounds in voice therapy.

Acknowledgements:

We thank for their contributions to the building of database (in alphabetical order): Dr. Zineida Bogulepova (Moscow, Russia), Dr. Amy Chan (Toronto, Canada), Prof. Philippe Dejonckere (Leuven, Belgium), Prof. Ilter Denizoglu (Izmir, Turkey), Prof. Ewa Niebudek-Bogusz (Lodz, Poland), Dr. Pedro Melo Pestana (Porto, Portugal) [22], and Dr. Herbert Steven Sims (Chicago, IL, USA). We thank Fondazione Cariplo, project Erisimo a Milano, 2017 for funding part of this work.

Supporting information:

S1 **Herbs for Voice database, excel file**.

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