Verbascum, Scrophulariaceae

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Verbascum species (common mullein) have been widely used in Spanish folk medicine to treat pathologies related to the musculature, skeleton, and circulatory, digestive, and respiratory systems, as well as to treat infectious diseases and organ-sense illnesses.

Keywords: Verbascum ; traditional knowledge ; validation ; flavonoid ; terpene ; inflammatory

1. Introduction

The genus Verbascum (Scrophulariaceae, Lamiales) comprises more than 300 Eurasiatic species. It is the largest genus of the family, and its origin is the center of the Eastern Mediterranean Basin. In the Iberian Peninsula, it is represented by 26 species [1]. In Spain, they are popularly named “gordolobos” (in English, common mullein), and the Spanish Inventory of Traditional Knowledge related to Biodiversity [2] has catalogued 10 species which have been used to treat a wide range of pathologies. These are Verbascum pulverulentum Vill. , V. sinuatum L., V. thapsus L., V. boerhavii L., V. creticum (L.) Cav. , V. dentifolium Delile, V. giganteum Willk. , V. lychnitis L., V. rotundifolium Ten. , and V. virgatum Stokes in With.

In order to realize the potential pharmacological application of these species, we must perform a deep analysis of their chemical compositions as a starting point to understand which phytochemicals could exert the medical actions described in the traditional knowledge. The chemical components of Verbascum spp. , and the biological actions attributed to these phytochemicals, can be found in the literature [3][4][5][6][7][8][9][10][11][12][13][14][15][16][17], with the correlation between the phytochemicals’ bioactivity and their traditional uses being a key point to validate their traditional ethnobotanical uses.

The aforementioned bibliographic prospection could be complemented by in silico approaches to demonstrate the phytochemicals’ affinities using molecular targets. The combination of bibliographic research and computer programming could provide a strong tool to approach the botanical bioactive compounds existing in Verbascum spp. with the medical uses collected by folk knowledge.

2. Use and application

2.1. Anti-Inflammatory Action of Verbascum

The role of biological molecules, such as inteleukins (ILs), lipooxygenase (LOX), cyclooxygenase (COX), nuclear factor κB (NF-κB), vascular endothelial growth factor (VEGF), matrix matalloproteinases (MMPs), and tumor necrosis factor (TNF), among others, with the onset of inflammation is well known as well as the link between inflammation and chronic diseases [18]. Therefore, the study of phytochemicals, able to block the action of the aforementioned molecules, is key in the search of new drug candidates to treat chronic diseases and other pathologies with a high inflammatory component.

Most of medicinal applications of *Verbascum* spp. collected from the folk knowledge, have in common an array of inflammatory processes; therefore, understanding the anti-inflammatory molecular mechanisms displayed by Verbascum phytochemicals is essential in order to explain most of its healing properties.

The results generated by our affinities studies show the affinity of flavones (apigenin and luteolin) and flavonols (quercetin, 3′-methylquercetin and kaempferol) by arachinodate-lypoxygenases (LOX), a group of enzymes implicated in the synthesis of eicosanoids, such as leukotriens (LTs), which are molecules with an essential role in cell signaling, being also implicated in inflammation and disorders, such as asthma, skin diseases, rheumatoid arthritis, allergic rhinitis, inflammatory bowel, cardiovascular diseases, cancer, and osteoporosis [19][20][21][22][23]. It is well-known the anti-inflammatory role of polyphenolic compounds [24], in which flavones and flavonols are included. The ability of these compounds to interfere with enzymes implicated in the synthesis of eicosanoids, such as LOX, is one of the molecular mechanisms underlying their anti-inflammatory properties, and the ability of quercetin and lutein to suppress LOX product synthesis has been scientifically proven [20]. Despite our in silico approach cannot provide information about the molecular

dynamic of phytochemical-target interaction, the affinity of flavones (apigenin and luteolin) and flavonols (quercetin, 3′-methylquercetin and kaempherol) for LOX, obtained by our in silico approach, is consistent with the scientific results found in the literature, in which the ability of quercetin and luteolin to suppress the formation of LOX products implicated in inflammation, such as LTs, is well demonstrated [20].

The polyphenolic compounds listed in **Figure 1** shared a cathechol partial structure, which could be responsible for uncoupling the catalytic cycle of LOX, due to its iron chelating and antioxidant properties [20].

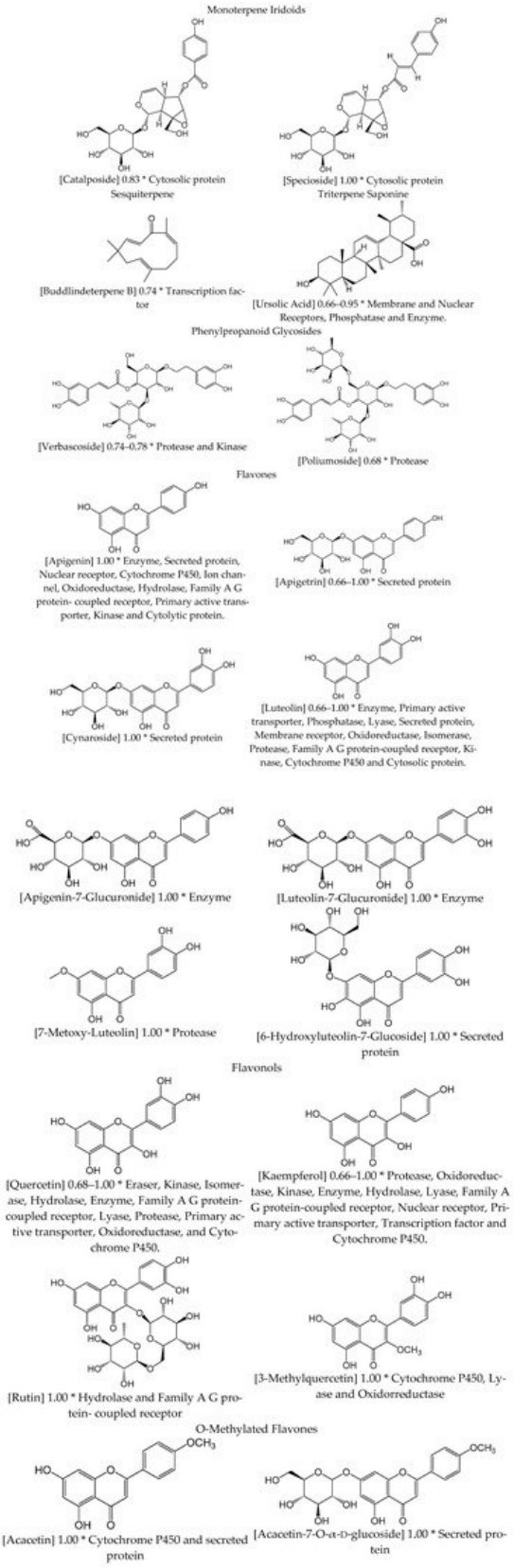

Figure 1. Chemical structures of Verbascum components with probability values and target class according to the SwissTargetPrediction classification. * Probability—target class.

Another interesting result obtained from our in silico studies has shown the affinity of luteolin, quercetin, and kaempferol for interacting with NOX4 (NADPH oxidase-4), an enzyme implicated in the generation of superoxide anions and other

downstream reactive oxygen species (ROS) [25]. For example, the protective role of luteolin against inflammation via the NOX4/ROS-NF-κB and MAPK pathways supports our findings and explains the anti-inflammatory action of mullein [26]. Compounds such as acacetin, apigetrin, and cynaroside have a high affinity to interact with the proinflammatory cytokines TNF-α and IL-2, which could also be related to their anti-inflammatory effects. In 2017, a paper from Hu et al. [27] demonstrated the anti-inflammatory effect of the flowers of Chuju (a medical cultivar of *Chrysanthemum morifolim* Ramat), which contain apigetrin and acacetin in their chemical composition [27]. A work of Zhao et al. (2014) [28] showed the ability of acacetin to block T-cell proliferation and IL-2 secretion, both essential to induce the inflammatory response underlying diseases such as rheumatoid arthritis and psoriasis [28]. The anti-inflammatory bioactivity of apigetrin has also been reported in an animal model of acute otitis media [29], which is a traditional use of Verbascum widely reported throughout the Iberian peninsula. Eventually, the anti-inflammatory effect of cynaroside has been demonstrated in a model of human periodontal ligament (hPDL) cells, a cell type essential in the maintenance of the periodontal tissues homeostasis, in which cynaroside has the ability to decrease the expression of pro-inflammatory cytokines, such as TNF-α, induced by LPS treatment [30].

Eventually, the in silico result, showing affinity between ursolic acid and the retinoic acid-related orphan receptor gamma (RORγ), a transcription factor essential for T helper cells differentiation, supported by experimental result showing an effective and selective inhibitory effect of this phytochemical over RORγ, could also explain the anti-inflammatory properties attributed to Verbascum spp [31].

2.2. Circulatory System Diseases

The most remarkable uses in this section are those related to circulation. The applications of these species against hemorrhoids and varicose veins are related to their local expansion processes in the peripheral circulation. This healing action can be explained by the presence of flavonoids, whose antioxidant and vasodilatory activities are associated with their protective cardiovascular action, widely referred to in the literature [32]. These compounds are common in aqueous extracts from the plants [33], so their presence is expected in many of the preparations recorded in Spanish traditional medicine and listed in **Table 1**. It has been reported that they are mainly used after being boiled and are then applied externally. The pathologies previously mentioned have also a local inflammatory component, therefore, the anti-inflammatory activity of common mullein, discussed in the previous section, could also underlie this group of healing remedies [23].

The antihypertensive use of *Verbascum* spp. reported in **Table 1** could rely on the interaction of Verbascum phytocompounds with the α-adrenergic receptors implicated in peripheral vascular resistance walls. On the one hand, the α-adrenergic antagonist activity of flavonoids could explain Verbascum's antihypertensive action [34]. On the other hand, the affinity of rutin to interact with the α2-adrenoreceptors obtained in our in silico assays, and its anti-hypertensive action reported in the literature [35], could contribute to the antihypertensive action of Verbascum reported from folk knowledge [36].

Table 1. Traditional uses of Spanish Verbascum.

Uses		*Vp*	*Vs*	*Vt*	*Vb*	*Vc*	*Vd*	*Vg*	*Vl*	*Vr*	*Vv*
Circulatory	Anti-hemorrhoidal	B/T	B/T	S/B/T	B/T			T	B	T	
	Leg treatment			B							
	Anti-hypertensive			I/B				I		B	
Digestive	Teeth pain, gumboil	B/T	B	B/T							T
	Digestive	I/B/T		B/T							
	Gastric ulcer/inflammation	B/T	I/B/T	B							
	Liver inflammation	I/B	T	I/B/T				I/B			
	Gallstone	I	I	I/B				I			
	Anti-diarrhoea	T	I	T							
	Constipation			B					E		

Uses		*Vp*	*Vs*	*Vt*	*Vb*	*Vc*	*Vd*	*Vg*	*Vl*	*Vr*	*Vv*
Respiratory	Hoarse, tonsillitis	B/T	I/T	I/B/T							
	Cold	B	I	I/B				I			B
	Cough, asthma, bronchitis, hemoptysis	B	I/B/M	I/B			I	I			B
Musculature & Skeleton	Anti-inflammatory (swelling)	B/T		I/B/T							
	Contusion, broken bones	I/T	T	I/B/T							
	Arthrosis, rheumatism		B/T	B/T				I		T	
Skin	Eczema, exanthema	B/T	B/T	T							
	Cysts and zits	T	T	I/B/T				T		T	
	Wounds, ulcers, burns	B/T	M/T	I/B/M/	T			T			
	Horsefly bite			M/T							
	Chilblain	B/T	B	B/T				B/T		B/T	
	Nail conditions			B/T							
Sense	Conjunctivitis	M	M	M							
	Otitis	B/M	M	B							
Infectious parasitic diseases	Diphtheria	T									
	Helminthiasis		B								
	Tuberculosis			I							
	Typhus			T							
	Mange			T							

(*Vp: V. pulverulentum; Vs: V. sinuatum; Vt: V. tapsus; Vb: V. boerhavii; Vc: V. creticum; Vd: V. dentifolium; Vg: V. giganteum; Vl: V. lychnitis; Vr: V. rotundifolium; Vv: V. virgatum).* Administration T: Topic; I: Infusion; B: Boiled; M: Maceration; E: Enem; S: Steam.

2.3. Digestive Apparatus

The digestive process begins with activity in the oral cavity, chewing, salivation, and swallowing. Therefore, oral health is essential for proper digestion. The employment of infusions and decoctions, of these plants by Spanish folk medicine, to treat tooth pain and gumboil could be related to the anti-inflammatory activity discussed above. The anti-inflammatory effect of common mullein could rely on the anti-inflammatory action of its phytochemical cynaroside which has been demonstrated to confer protection against the inflammation underlying the periodontitis [30].

Other applications include for digestive problems, gastric ulcer, or inflammations in different parts of the digestive system (stomach, liver, gallbladder), for which there are treatments described in the traditional Spanish uses of the plant (**Table 1**). One study indicates the protective effect of ursolic acid against hepatotoxicity in mice [37].

In addition, some of these proteins are specifically related to the physiology of the gastro-intestinal tract. Salivary amylases help to break down food into its molecular components. Parietal cells in the stomach release various acids, pepsins, and enzymes, including gastric amylase, to achieve partial digestion and obtain chemo (semi-fluid and semi-digested mass). Acids also neutralize salivary amylase, favoring gastric intervention. After about an hour, the chimo is pushed into the duodenum, where acidity acquired in the stomach stimulates the release of the hormone secretine. The pancreas then releases hormones, bicarbonate, bile, and numerous pancreatic enzymes, such as lipases (P04054), and those of the lipidic metabolism, such as aldoreductases and most of the ones consigned in the “Enzyme” file. These are related to glucose conversion in NADPH-dependent sorbitol, the first step in the poliol pathway of glucose metabolism [38]. Afterwards, thanks to bicarbonate, the acidity of the chimo is changed into an alkaline form, allowing the better degradation of food and also creating a hostile environment for bacteria that survived the passage to the stomach. This process can be carried out effectively and smoothly if the enzyme system is healthy; otherwise, careful supplementation is required [39].

More difficult to validate, however, is the use related to defecation processes. These species have been used as both astringents and laxatives, and the only possible explanation for the traditional use of these plants is that in the first case, diarrhea (for which infusions are taken) has some infectious origin and causes inflammation. In the second case, where enemas are used because of the evacuating effect achieved by the mechanical action of water, this is favored by the presence of triterpene saponins, which have the ability to produce soapy solutions.

2.4. Respiratory Diseases

Respiratory tract pathologies treated with mullein have different etiologies (hoarseness, tonsilitis, colds, coughs, asthma, bronchitis, and even hemoptysis) and treatments, but all have a common feature: the development of inflammatory processes. Besides this, in many cases, fever and cough are displayed. The relief properties of mullein could be explained by its antitussive and expectorant activities, which could be justified by the presence of mucilages in these species [40] which exert demulcent activity [41].

Ursolic acid is one of the most promising substances of biological origin for antimicrobial therapy. It has been identified as a phytochemical inhibitor of the main protease of COVID-19 using molecular modelling approaches [42][43][44]. Other potential phytochemicals of Verbascum spp., which could be useful to treat COVID-19, are the flavonoids apigenin, luteolin, and quercetin, which have been shown to be replication inhibitors of other coronaviruses [45].

Since, in severe COVID-19 patients, an elevation of pro-inflammatory cytokines occurs, also known as “cytokine storm”, that is responsible of deteriorating their health conditions, the search of drugs able block target this “cytokine storm” and suppress the exacerbated inflammatory response is key in the treatment of the complications associated to the disease [46]. Our in silico results have evidenced affinity between mullein phytochemicals (Flavones and O-metilated flavones) and pro-inflammatory cytokines (IL-2 and TNF-α), molecules implicated in inflammatory processes related to the respiratory system and COVID-19 [47][48][49]. The previously validated anti-inflammatory activity of Verbascum components also supports the potential use of the extracts from the plants tackled in this review to achieve the desire anti-inflammatory action requested to prevent and treat COVID-19 acute clinical profile. The employment of natural compounds with immunosuppressant properties could be useful as adjuvants to ameliorate the inflammatory process triggered by the out-of-control immune response which could be fatal for the patient, even causing death [50].

Our hypothesis suggesting the employment of Verbascum flavonoids as promising COVID-19 treatment is extensively supported by the existing literature which includes a large number of works using in silico and in vitro approaches which demonstrate the ability of flavonoids to interfere with the viral infection or to prevent/ameliorate the COVID-19 disease effects. Among SARS-CoV2 targets blocked by flavonoids $3CL^{pro}$ (the protease responsible of processing the two polyproteins firstly translated after viral entry) can be highlighted due to its pivotal role in the initiation and progression of the viral cycle and the lack of its human homologue. Apigenin, luteolin, kaempferol, and quercetin are able to inhibit the proteolytic activity of $3CL^{pro}$, quercetin being the most effective. The ability of these phytochemicals to interact with $3Cl^{pro}$ could be due to the ability of the two phenyl groups of flavonoids to interact with the protease substrate binding pocket [51]. Another target is the RNA-dependent RNA polymerase (RdRp) responsible or virus genome replication. The RdRp activity, and therefore the viral replication, is affected by high Zn^{2+} levels and quercetin can act as Zn^{2+} ionophore facilitating the influx of Zn^{2+} into the cell [52]. The last molecular target to deal with SARS-CoV-2 infection is to block the interaction between the SARS-CoV-2 Viral Spike Protein (S) and its cellular receptor, the Angiotensin Converting Enzyme-2 (ACE2) protein, responsible of viral entry. In silico experiments have shown the capacity of two flavonoids (quercetin and luteolin) to block this process [53][54].

The main challenge found in the use of flavonoids, such as quercetin, with a widely supported antiviral action is the poor oral bioavailability due to its reduced absorption and biotransformation during digestion [55][56]. This issue can be tackled through alternative administration ways, such as nasal spray [57] or phytosomes [58].

2.5. Musculature and Skeleton

The use of analgesic, anti-inflammatory, and/or antipyretic drugs is very common in treating a wide range of medical conditions in current clinical pharmacology. Traditional medicine has also used many plants with identical purposes, such as the *Verbascum* spp. studied here. The applications listed in **Table 1** extracted from the Spanish National Inventory include a wide spectrum of remedies to treat osteoarthritis, rheumatism, hand crack, kneeache, gout footache, contusions, and even broken bones, all of them characterized by the onset of inflammation and pain. The main aspects considered in the preceding paragraphs have already been discussed within inflammation section.

Pain has been defined by the IASP (International Association for the Study of Pain) as an unpleasant sensory and emotional experience associated with or resembling that associated with actual or potential tissue damage [59]. The phenomenon is a multidimensional entity and nuanced elements of pain are not easy to apprehend when pain is measured with the standard qualitative metrics [59]. From a biochemical and molecular biology point of view, the relationship of certain proteins with painful effects is well known [60], although the potential utility of proteomics to investigate pain management has just started to be considered. Cytochrome P450 [61], gyoxalase I [62], myeloperoxidase [63], and kinases [64] are proteins involved in the physiopathology of pain. The in silico study points to the great affinity of phytocompounds of these vegetables—particularly quercetin, kaempferol, apigenin, and luteolin—with these proteins were summarized.

Osteoarthritis, one of the illnesses treated with common mullein by Spanish traditional medicine, is characterized by the degradation of cartilage, inflammation, and osteophyte formation in joints. Metalloproteinases are directly related to the onset of this medical condition due to their ability to proteolyze the extracellular matrix [65]. The affinity of some Verbascum phytochemicals (verbascoside, poliumoside, luteolin, quercetin, and kaempferol) for metalloproteinases could explain the traditional employment of mullein in osteoarthritis treatments. This notion is supported by a recent work which suggests the employment of verbascoside to treat osteoarthritis [65]. The employment of an ethanolic extract of Moussonia deppeana (high verbascoside content) shows an anti-edematous action in an experimental model of arthritis [66]. The ability of quercetin to reduce the severity of rheumatoid arthritis has also been demonstrated in vivo [67]. Another molecular mechanism, implicated in rheumatoid arthritis, is the invasion of fibroblast-like synoviocytes (FLS), which is responsible for cartilage destruction. Again, the metalloproteinases are involved in FLS invasion and kaempferol is able to reduce FLS migration and invasion both in vitro and in vivo [68].

A similar reasoning can be found regarding fever. Antithermic action is related to TNF-α secreted proteins [69] (P01375, **Table A2**), which have shown an in silico affinity with Verbascum flavones (6-hydroxyluteolin-7-glucoside, apigetrin, and cynaroside) and O-metilated flavones, such as acacetin-7-O-α-d-glucoside.

2.6. Skin and Sense Organs

The topical dermatological use of various extracts (infusion, boiling, maceration) from these plants for the treatment of occasional or repetitive local eruptions (cysts, zits, eczemas, exanthemas), accidental or more serious conditions (wounds, ulcers, burns, bites), and even eye or ear inflammations are justified by their anti-inflammatory power reported throughout this manuscript.

The employment of common mullein to treat otitis could be explained by the presence of apigetrin in its chemical composition. We have shown the high affinity of apigetrin for TNF-α and IL-2 (P01375 and P60558, respectively), both belonging to the cytokine family and implicated in inflammatory processes. This hypothesis is supported by a recent work which demonstrates the healing effect of apigetrin in otitis media due to its ability to suppress inflammation and oxidative stress. Treatment with apigetrin reduces mucosa thickness, inhibits the inflammatory response by downregulating neutrophils and macrophages, and reduces ROS generation, eventually alleviating otitis [29].

2.7. Other Uses

Other popular uses, such as in the treatment of infectious diseases and parasitosis (diphtheria, helminthiasis, tuberculosis, typhus, and mange), require a direct validation that is difficult to explain with the data currently available. Indirectly, all the anti-inflammatory actions discussed throughout this work need to be taken into consideration.

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