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Study Of Bulgarian Plant Extracts Effect on Three Bacterial Sialidases

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Abstract

Since sialidase is a pathogenicity factor in certain microbes, it is important to inhibit it as this could have both preventative and therapeutic impacts on a number of diseases. Viral sialidase inhibitors have previously produced significant effects, but there are few experiments of the same kind on bacterial enzymes. The ability of some extracts from Bulgarian plants to inhibit the sialidases of *Vibrio cholerae* non-O1, *Arthrobacter nicotianae*, and *Oerskovia paurometabola* was investigated. Extracts from *Rosa damascena*, essential oil from *Origanum vulgare* ssp. *hirtum* and acetone exudate from *Helichrysum arenarium* were found to have an inhibitory effect on the studied enzymes. The influence of the extracts on bacterial sialidase activity was investigated for the first time. It was found that 5% Triton X-100 used for extract dilution has inhibitory effect on sialidase produced from *O. paurometabola* (76%), from *V. cholerae* non-O1 (30%) and has no effect on *A. nicotinae*.

Keywords: bacterial sialidases, inhibitors, *Rosa damascene, Origanum vulgare*

Резюме

При някои микроорганизми сиалидазите са патогенен фактор. Ето защо намирането и изучаването на нови инхибитори на тези ензими представлява интерес за учените, тъй като те могат да имат както превантивно, така и терапевтично въздействие върху редица болести. Докато инхибиторите на вирусни сиалидази са подробно проучени през годините и е ясен техния значителен ефект, то подобни опити проведени с инхибитори на бактериални сиалидази са малко на брой. В настоящата работа е изследвана способността на някои екстракти от български растения да инхибират сиалидази от *Vibrio cholerae* non-O1, *Arthrobacter nicotianae* и *Oerskovia paurometabola*. Инхибиращ ефект спрямо трите изследваните ензима оказват екстракти от *Rosa damascena*, етерично масло от *Origanum vulgare* ssp hirtum и ацетонов ексудат от *Helichrysum arenarium*. Влиянието на избраните екстракти от български растения върху бактериална сиалидазна активност е изследвано за пръв път. Установено е, че 5% Тритон Х-100, използванеза разтваряне и разреждане на екстрактите има потискащ ефект спрямо сиалидази от *O. paurometabola* (76%) и *V. cholerae* non-O1and (30%) и не оказва ефект спрямо ензима от *A. nicotinae*.

Introduction

Sialidases, also known as glycoside hydrolases (EC 3.2.1.18), acetylneuraminyl hydrolases, exo-alpha sialidases, or neuraminidases, play a role in the metabolism of humans and animals belonging to the *Deuterostomata lineage*. Additionally, these enzymes are found in certain parasitic or commensal microorganisms, such as viruses, bacteria, fungi, and unicellular eukaryotes. Their function involves the cleavage of terminal sialic acid residues from various complex sialosides, encompassing glycoproteins, glycolipids, polysaccharides, and polysialic compounds. This enzymatic activity results in the liberation of free sialic acid as a byproduct (Schauer and Kamerling, 2018).

Microbial sialidases were found to activate host chemokine production and neutrophil homing to the site of infection, to increase the production of the interleukin IL-8 in lung epithelial cells, to enhance neutrophil immune activation and induce an inflammatory response (Banerjee *et al*., 2010), etc. Sepsis, a common cause of death in hospitalized patients, is exacerbated by bacterial sialidases, interrupting an inhibitory circuit in dendritic cells that arrest the immune response when host

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cells are destroyed. Blocking sialidase activity results in a substantial limitation of the inflammatory response and subsequent mortality (Paulson *et al*., 2011). Sialidase was found to promote biofilm formation and capsule synthesis (Li *et al*., 2012). In *Vibrio cholerae* it supports the action of cholera toxin (Kaisar *et al*., 2021). Prevention of all these effects is possible with the inhibition of microbial sialidases, therefore they are considered potential drug targets.

Various types of sialidase inhibitors have been described in the literature, depending on which element or step of the sialidase action they suppress. Based on their inhibition mechanisms, sialidase inhibitors include (i) transition-state analog inhibitors, (ii) mechanism-based inhibitors, (iii) suicide substrate inhibitors, (iv) product analog inhibitors, and (v) allosteric site inhibitors (Sharapova *et al*., 2018; Keil *et al*., 2022).

Plants are a source of medicinal agents used by people throughout history, from ancient times to the present day. They are also a source of potential enzyme inhibitors, as natural products from plant extracts are of diverse stereoisomerism and often exhibit high specific biological activity (Keil *et al*., 2022). Reliable inhibitors of *Trypanosoma cruzi* trans-sialidase were found among natural flavonoids and anthraquinones (Arioka *et al*., 2010). Caffeic acid was found to exert inhibitory activities on viral sialidases (Xie *et al*., 2013). The development of selective inhibitors against bacterial sialidases has not been this successful, despite that crystal structures are available for many of them (Slack *et al*., 2018). The active site of sialidases is highly conserved and provides a uniform catalytic mechanism. Nevertheless, different sialidases, especially those of bacterial origin, have their unique features and this allows the search for specific inhibitors for each of them. The aim of our study is to test a set of plant extracts for potential inhibitory action on three bacterial sialidases that have not been studied in this aspect yet.

Materials and Methods

Bacterial sialidases

Three bacterial strains were used as producers of sialidases: *A. nicotianae* (AN), *V. cholerae* non-O1 strain V13 (NBIMCC 8716) (V13), and *Oerskovia paurometabola* strain O129 (NBIMCC 9093) (O129). The enzymes were previously purified, and for *A. nicotianae* they were stored as a lyophilized powder (Abrashev *et al*., 2005), while for O129 and V13, they were stored as phosphate-buffered saline (PBS) solution at -20ºC (Eneva *et al*. 2015; Eneva *et al*., 2022). Before performing the inhibition test, the enzymes were standardized to solutions with 10 U/mL sialidase activity by dilution in PBS.

Plant material

In the present study, the plant material of the studied species *Helichrysum arenarium* (L.) Moench flower, aerial parts of *Origanum vulgare* subsp. *hirtum,* (Link) Ietsw., *Thymus pulegioides* L., *Artemisia santonicum* L. were collected from their natural populations. Material of *R. damascena* Mill (petals) was kindly provided by Dr. Ani Dobreva from the Institute for Roses and Aromatic Plants, Kazanluk, Bulgaria.

Preparation of extracts and fractions

The ethanol extract was prepared by classical maceration with 80% ethanol for 24 h, filtration, and evaporation to dryness. Ethyl acetate fraction was obtained from crude ethanol extract by liquid/liquid extraction, using distilled water and ethyl acetate. Acetone exudates were prepared from air-dried, not-ground plant material by rinsing with acetone for a few minutes to dissolve the material accumulated on plant tissue surfaces (Nikolova *et al*., 2023). Essential oil was extracted from *O. vulgare subsp. hirtum* by water distillation performed using a Clevenger apparatus (Nikolova *et al*., 2021).

Enzyme assay

Sialidase activity was determined by the method of Uchida *et al*. (1977) also known as the thiobarbituric acid method. Glucomacropeptide (GMP) obtained from cheese was used as a substrate (Abrashev *et al*., 1980). Separate 200-µl enzyme samples were mixed with 200 µl of each of the tested compounds at different concentrations. Extracts of *R. damascene* and *T. pulegioides* L were dissolved in 5% dimethyl sulfoxide (DMSO) to final concentration respectively (0.5; 1.25; 2.5 mg/mL). *O. vulgarae, H. arenarium*, and *A. santonicum* L extracts were dissolved in 5% Triton X-100 (12, 25, 50% final concentration). Control 200-µl enzyme samples mixed with 200 µl 5% DMSO or 5% Triton X-100 in PBS were also prepared. The absorbance of samples and controls was measured on a UV-VIS 75 at λ=551nm. The amount of sialic acids released was determined as the difference (ΔE) in the extinctions of the sample and control was plotted on a standard curve, created using sialic acid as a standard. One unit of sialidase activity is defined as the amount that releases 1 µmol of N-acetylneuraminic acid (Neu5Ac) for 1 min at 37°C using GMP as a sub-

strate. Results were expressed as a percentage of inhibition. All experiments were performed in triplicates to ensure accuracy of the results and the data has been presented as mean ± standard deviation.

Results and Discussion

Some of the plant exudates and essential oils tested in our study showed significant sialidase inhibition. Both the ethanol and its ethyl acetate fractions of *R. damascena* significantly inhibited the three sialidases tested. The ethyl acetate fraction in the maximum tested concentration (2.5 mg/mL) suppressed *A. nicotianae*, O129, and V13 sialidases 49, 83, and 57%, respectively The ethanol extract in the same concentration manifested stronger inhibition - 78, 86, and 80% (Fig. 1).

The strong inhibitory effect of the ethanolic extract of *R. damascena* is comparable with the anti-sialidase activity of some plants used in Chinese traditional medicine. Ethyl-acetate fractions of *Pyrola calliantha* and *Geranium strictipes* demonstrated 79% and 84.6% inhibition against influenza virus sialidase (Yang *et al*., 2016). Roses are traditionally used as ornamental plants because of their beautiful appearance and pleasant aroma. Bulgaria has traditions in the production of rose oil of the best quality together with countries like Turkey and Morocco. Apart from its importance for the perfume industry, *R. damascena* also has valuable pharmacological and medicinal properties including anti-HIV, antibacterial, antioxidant, antitussive, hypnotic, antidiabetic, and relaxant effects on tracheal chains (Boskabady *et al*., 2011). Roses are rich in phenolic compounds which are mainly responsible for their medicinal importance (Yang *et al*., 2016). Although the inhibition of sialidases is not among the known properties of *R. damascena* extracts, our results have a logical explanation, considering the abundance of phenolic derivatives contained in these plants, having in mind that similar compounds are well-known sialidase inhibitors (Keil *et al*., 2022). Moreover, plant extracts from *R. damascena* have proven inhibitory activity against alpha glycosidases, enzymes with mechanisms of action similar to that of sialidases (Akram *et al*., 2020).

When commenting on the results regarding the influence of plant extracts dissolved in Triton X-100, the inhibitory effect of the solvent *per se* on the enzymes of O129 and V13 should be taken into account. The results in Fig. 2 show that the substance in which the extracts were dissolved, namely Triton X-100, has no inhibitory effect on sialidase isolated from *A. nicotinae*, but it inhibits the activity of enzymes isolated from O129 and V13.

The effect of the *O. vulgare* ssp. *hirtum* essential oil on the AN sialidase (85% inhibition) is interesting, since most tested substances do not have a strong effect on this sialidase (Fig. 2). The other two enzymes are not suppressed. Most likely, the inhibitory effect is due to the rich range of natural chemical compounds contained in oregano plants. The chemical profile of white oregano essential oil from Bulgarian natural populations of the species and cultivated areas showed that it belongs to the carvacrol chemotype, as its amount varies in different years and origins. The other two main components and precursors in the synthesis of carvacrol and thymol are p-cymene and γ-terpinene (Baycheva and Dobreva, 2021; Nikolova *et al*., 2021).

Along with antioxidant and antimicrobial activities, the *O. vulgare* essential oils exerted a suppressive influence against some enzymes like acetylcholinesterase, butrylcholinesterase, α-amylase and α-glucosidase (Georgiev *et al*., 2022).

T. pulegioides L. exudate showed moderate inhibition against the three sialidases, with the percentage of inhibition against *A. nicotianae* enzyme being the most pronounced at the concentration of 2.5 mg/mL (55% compared with 27% and 20%

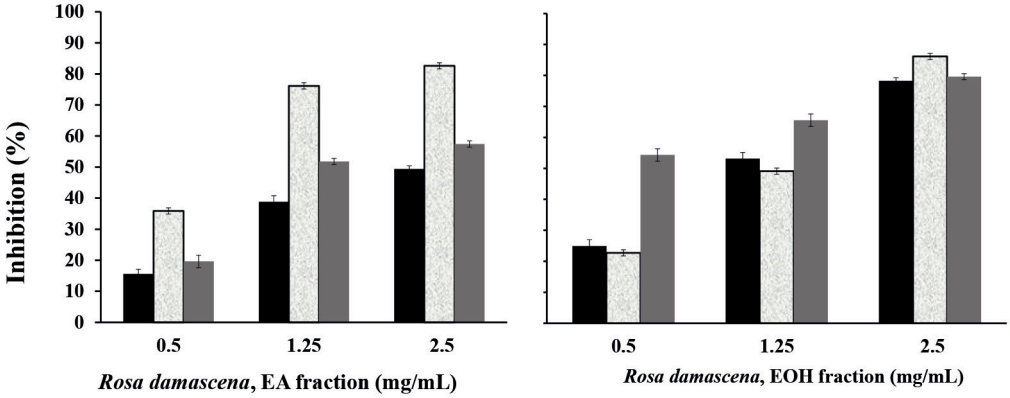

Fig. 1. Inhibition effect of extracts from *R. damascena* on bacterial sialidase from *A. nicotianae* (light gray); *O. paurometabola* (dark grey); *V. cholerae* (black)

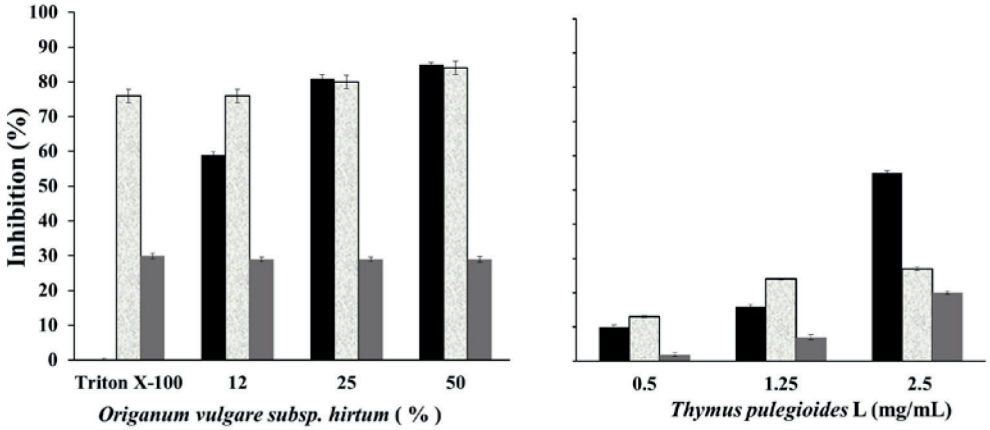

Fig. 2. Inhibition effect of extracts from *O. vulgare subsp. hirtum* and *T. pulegioides* on bacterial sialidase from *A. nicotianae* (light gray); *O. paurometabola* (dark grey); *V. cholerae* (black)

for O129 and V13 enzymes respectively). Thyme is widely used in traditional medicine as a remedy against colds, for internal applications to treat gastrointestinal and respiratory diseases, to regulate blood pressure, etc.

T. pulegioides is rich in phenolic acids, especially rosmarinic acid, which largely accounts for the medicinal importance of this plant. It was established that aqueous decoctions and hydro-ethanolic extracts of *T. pulegioides* have an anti-proliferative and antoxidant activity. These extracts are characterized by a moderate anti-aging effect, and strong neuroprotectivity and are able to suppress acetylcholinesterase (80% inhibition) and tyrosinase (94% inhibition) (Taghouti *et al*., 2018).

The findings from the investigation into the impact of extracts derived from *H. arenarium* (L.) and *A. santonicum* on the activity of three bacterial sialidases are shown in Fig. 3. The exudates from *H. arenarium* and *A. santonicum* did not affect the activity of the enzyme isolated from *A. nicotinae* at all concentrations tested. Taking into account the effect of the solvent on the other two enzymes, it can be noticed that the exudate from *H. arenarium* (L.) does not inhibit the enzyme isolated from O129 and slightly suppresses the activity of the enzyme isolated from V13. Exudate from *A. santonicum* (L.) demonstrated a relatively low inhibition effect on the enzymes isolated from O129 and V13.

The biological activities of aromatic plants from *Artemisia* and *Helichrysum* species have been used in traditional medicines, for many years. Also, they are widely used as food supplements, in pharmacy and cosmetology. It was found that the methanolic extract of *A. santonicum* exhibits antioxidant and antimicrobial activity against different bacteria and fungi including food-associated microorganisms (Nikolova *et al*., 2021) According to Judzentiene *et al*. (2022), *H. arenarium* (*Helichrysi flos*) inflorescences have been used in European herbal medicine for their choleretic, cholagogue, diuretic, anti-inflammatory, hepatoprotective, digestive, anti-atherosclerotic, detoxifying, antimicrobial, antioxidant, cytogenetic, anti-cancer, and anti-hyperglycemic properties. They are rich in flavonoids, essential oils, fatty acids**,** carotenoids, phytosterols, bitter substances, phenolic compounds, vitamins, and mineral salts.

Conclusions

The treatment of various microbial infections

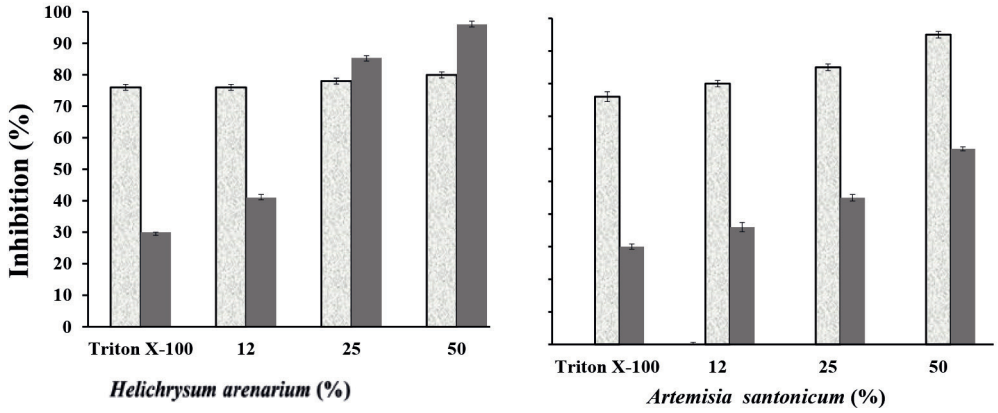

Fig. 3. Inhibition effect of extracts from *H. arenarium* (L.) and *A. santonicum* on bacterial sialidases from *O. paurometabola* (light gray) and *V. cholerae* (black)

can benefit significantly from strategies focused on inhibiting bacterial sialidase activity by plant extracts. In our study, extract from *R. damascena* had a strong inhibitory effect on all three studied enzymes. *O. vulgare* ssp. *hirtum* essential oil strongly inhibited the *A. nicotianae* sialidase. The specific effect that most of the extracts gave on each of the enzymes, indicates that specific inhibitors for individual bacterial sialidases can be sought among such natural products. Future studies on the still poorly explored kinetics of bacterial sialidase inhibition by extracts from Bulgarian traditional plants will provide useful information on their potential application as antibacterial and therapeutic substances of natural origin. Our findings prove that the three tested extracts from Bulgarian essential oil plants are able to inhibit bacterial enzymes that represent factors of pathogenicity and this makes them promising for future application in medicine and pharmacy.

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