Capturing antibacterial natural products with *in silico* techniques

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Abstract. The aim of the present study was to index natural products in order to facilitate the discovery of less expensive antibacterial therapeutic drugs. Thus, for modeling purposes, the present study utilized a set of 628 antibacterial drugs, representing the active domain, and 2,892 natural products, representing the inactive domain. In addition, using the iterative stochastic elimination algorithm, 36 unique filters were identified, which were then used to construct a highly discriminative and robust model tailored to index natural products for their antibacterial bioactivity. The area attained under the curve was 0.957, indicating a highly discriminative and robust prediction model. Utilizing the proposed model to virtually screen a mixed set of active and inactive substances enabled the present study to capture 72% of the antibacterial drugs in the top 1% of the sample, yielding an enrichment factor of 72. In total, 10 natural products that scored highly as antibacterial drug candidates with the proposed indexing model were reported. PubMed searches revealed that 2 molecules out of the 10 (caffeine and ricinine) have been tested and identified as showing antibacterial activity. The other 8 phytochemicals await experimental evaluation. Due to the efficiency and rapidity of the proposed prediction model, it could be applied to the virtual screening of large chemical databases to facilitate the drug discovery and development processes for antibacterial drug candidates.

Introduction

Today we are witnessing a notable increase of bacterial resistance to a wide range of antibiotics, reported worldwide. This has stimulated intensive efforts to search for new antibiotics, as well as for valued antibacterial agents, that can be utilized to treat infectious diseases (1,2). Severe infections caused by bacteria that have become resistant to regularly used antibiotics have become a major global healthcare problem in the twenty-first century (3-5). Antibiotic resistance, connected with Iatrogenesis, and an increasing number of hospital-acquired infections, mainly in critically ill and immunosuppressed patients, has now become established in the community, causing severe infections that are difficult to diagnose and treat (6). Bacteria have developed resistance to most classes of antibiotics that have been discovered so far (7). Several genes, many of which can be transferred between bacteria, encode antibiotic resistance. New resistance mechanisms are continually being described, and new genes and vectors of transmission are identified on a regular basis.

The molecular mechanisms by which bacteria have become resistant to antibiotics are varied and complicated (8,9). The most common type of bacterial resistance is acquired and transmitted via horizontal gene transfer (the antibiotic resistance genes are loaded on plasmids, which can act as vectors that transfer these genes to other members of a bacterial species or genus) (10). New mechanisms of resistance have affected several classes of antibiotics, leading to the aberrance of multidrug-resistant bacterial strains, some known as *superbugs* (7). The overuse and/or misuse of antimicrobial agents in patient clinics, in hospitalized patients, and in the food industry are the principal factors leading to antibiotic resistance (7). In recent years, the number of new antibiotics licensed for human use in different parts of the world has decreased. This development of drug resistance to frequently used antibiotics by human pathogens has driven the search for new antimicrobial chemicals, chemotherapeutic agents, and agrochemicals that may combine higher antimicrobial efficacy with lower toxicity, and minimize a negative impact on the environment.

For ages, various cultures around the world have used medicinal plants to treat or cure all sorts of diseases. The natural products (NPs) of plants, mostly responsible for plant pigmentation and flavor, are produced as secondary metabolites and serve as defense mechanisms against bacteria, insects, and herbivores. NPs have been adjusted to interact with biological systems via a long natural selection process (11,12). Consequently, they have long been a basis of therapeutics (13), and most of today's marketed drugs are natural-based products or their derivatives (14). This supports the claim that natural-based products are essentially better accepted by the body than synthetic

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Abbreviations: ISE, iterative stochastic elimination; MCC, Matthews correlation coefficient; EF, enrichment factor; vHTS, virtual high-throughput screening; TP, true positive; FP, false positive; MBI, molecular bioactivity index

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chemicals and have a better chance to be successful drugs. (15) During the 1980s and 1990s, following the introduction of combinatorial chemistry and high throughput synthesis, nature became a less important source of drug candidates in drug discovery projects. However, even though drug research global expenditures have more than doubled since 1991, the number of new drug entities that are approved annually by the Food and Drug Administration in the U.S. (FDA) is dropping off; in 2016 only 23 therapeutic new chemical entities were approved, the fewest in almost last five decades and below statistical expectations. (16) To remedy this situation, the main players in the field of drug discovery and development (the pharmaceutical industry and academic researchers) have returned to searching for new drugs in Nature's pantry (17,18).

Since the discovery and development of a new drug is a long and costly process, we use computer methodologies to facilitate the identification of new lead compounds and to optimize drugs in clinical use (19,20). Structural-based (21-23) and ligand-based (19,24-27) computerized methods are used increasingly for the construction of models that can predict the bioactivity of molecules and for the *in silico* screening of chemical databases. For modeling process, it is necessary to have sets of active and inactive chemicals and an optimization technique. We assume that active ligands have common features that are not easily detectable if only a small number of active ligands are used (28). For this reason, usage a larger number of active and inactive ligands in the modeling process ensure that more significant and robust conclusions can be obtained regarding the properties of these ligands. As well, it is worth noting that including compounds in the sample of inactive chemicals that possess properties similar to those of the compounds in the screened chemical database increases the applicability of the prediction model to virtual screening. Since a large number of physicochemical properties should be considered during the modeling process, we need extraordinary optimization techniques that are capable of overcoming the limitations of the combinatorial nature of the molecular bioactivity-indexing problem. During the last decade, we developed a new optimization algorithm, termed *iterative stochastic elimination* (ISE), that is able to scan multi-dimensional space and detect the best solutions (the global minimum and the best set of local minima) (29-31). We have applied this novel algorithm to several ligand-based problems (28,32). In this research, we used the ISE algorithm to build the filters, and the MBI equation to construct the model for indexing natural products for their potential antibacterial activity. Analysis of the filters enabled us to map physicochemical properties/descriptors that might contribute significantly to antibacterial activity.

Materials and methods

We used a set of 628 anti-bacterial drugs (collected from the Comprehensive Medicinal Chemistry Database and the literature) to represent the active domain for modeling and bioactivity-indexing purposes. The list of antibacterial drugs (documented in SMILES format and/or by their common names) could be supplied upon request from the corresponding author. Another set, composed of 2,892 NPs, was selected to represent the inactive domain. The database of NPs was prepared by collecting phytochemicals isolated from more than 800 diverse plants, spread worldwide, that can be obtained from AnalytiCon Discovery GmbH (Potsdam, Germany; www.ac-discovery.com). To construct an accurate predictive model, it is necessary to use sets of molecules that cover the space of the properties of the molecules in the screened database. As well, we had to select, as the inactive set, molecules with the same 'property space' as the screened molecules. Fig. 1A and B show the diversity within the antibacterial drugs and the natural products database, respectively.

Categorizing the 628 antibacterial drugs based on their own mechanism of action might enable us to construct different models, depending on the category of active molecules. However, we prefer to use the entire set of the 628 antibacterial drugs as active set and not to categorize them based on their mechanism of action in order to obtain more robust model (due to utilizing big and diverse set of active ligands) and to be more focused on the indication (antibacterial) and not on the biological target. From our past experience, by using the ISE-based indexing technique, we were successful in constructing discriminative filters and in proposing highly predictive models when applied for general properties such as drug likeness (28), antidiabetic (25), anti-inflammatory (33), anticancer (27).

MOE software, v2009.10 (www.chemcomp.com) was used to calculate the physicochemical properties (termed *descriptors*) of all the chemicals in the two databases. The calculated descriptors included molecular weight, logP, H-bond donors/acceptors, solubility, total charge and charge distribution, the types and number of atoms, and so forth (www.chemcomp.com/journal/descr.htm). Both databases of active/inactive ligands were divided into two-thirds for the training set and one-third for the test set. An in-house random picking module performed the split.

The cheminformatics version of the ISE algorithm (28) was used to construct models tailored to index phytochemicals for their potential antibacterial activity. Through efficient searching of the multivariable space, we constructed a large set of filters tailored to distinguish between antibacterial and inactive ligands. Each filter is composed of a certain sets of descriptors, and each is limited to an assigned range. The process of filter selection and construction is highly complex and requires the use of a highly efficient optimization algorithm, since the descriptors generally interact with each other, and changes in the range of one descriptor can have an effect on the best range of another descriptor. In order to arrive at the best set of filters, the optimization process ought to consider all descriptors in the set simultaneously. Fig. 2 describes the main items in the modeling process. For detailed descriptions of the ISE optimization technique and its utility in choosing sets of descriptors and optimizing their ranges, see our previously reported research studies (32).

Results and Discussion

Structural similarity analysis was conducted to assure that both sets of active/inactive chemicals were not biased and displayed adequate diversity. As shown in Fig. 1, both sets of chemicals are diverse. The 341 antibacterial drugs and 1,119 natural products had a diversity of less than 0.5 in terms of the structural Tanimoto index. As well, analysis of

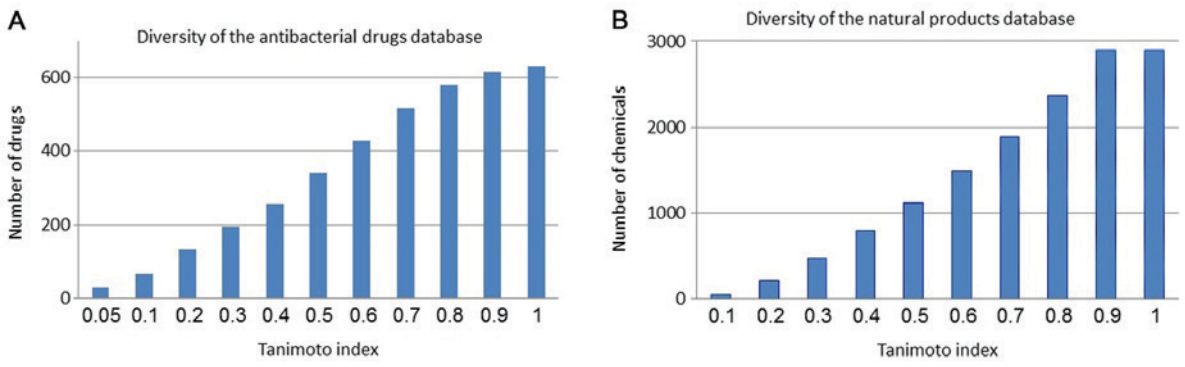

Figure 1. Diversity of the (A) antibacterial set of drugs and the (B) natural products database.

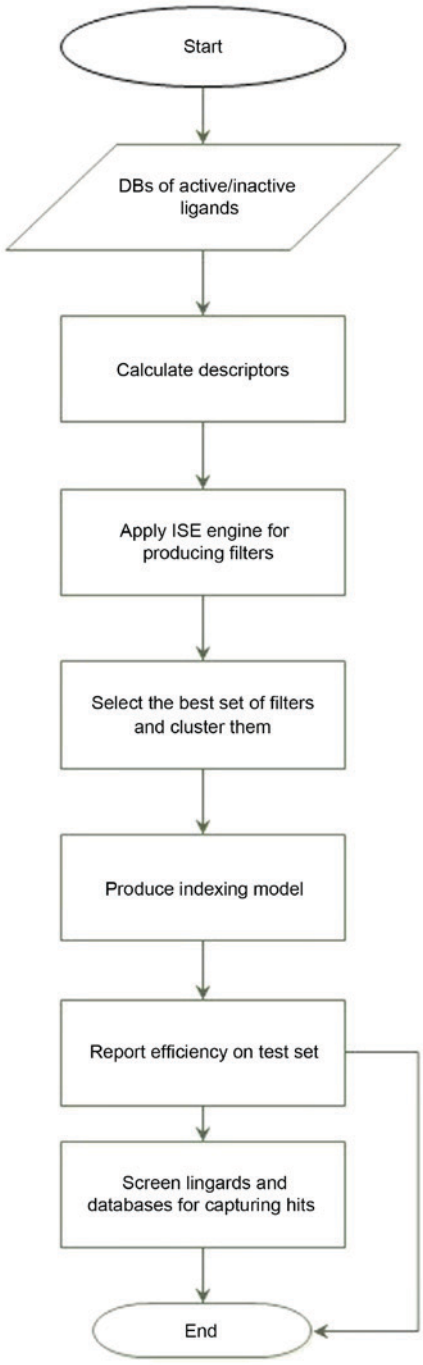

Figure 2. Flowchart for the ligand-based modeling process. ISE, *iterative stochastic elimination*; DB, databases.

the physicochemical properties noted that 71.5% of the antibacterial drugs conformed to Lipinski's rule of 5 (ROF), and 61% conformed to Oprea's rule for lead-likeness (34) (Fig. 3). Fig. 3 displays the distribution plots for the physicochemical properties of the antibacterial drugs related to Lipinski's ROF and Oprea's rule for lead-likeness. The median is around 396 for the molecular weight; 1.2 for logP; 7-8 and 2-3 for the hydrogen bond acceptors and hydrogen bond donors, respectively (Fig. 4).

The aforementioned filter-based indexing technique was utilized to launch an *in silico* prediction model capable of discovering novel antibacterial drug candidates. It was built using a set of 628 antibacterial drugs to represent the active domain, and a set of 2,892 natural products to represent the inactive domain. It is worth noting that a few chemicals out of the 2,892 natural products might have had antibacterial activity, but the effect of that on the quality of the prediction model was anticipated to be negligible (28,32). The optimization technique used to construct the filters was the ISE algorithm. Thirty-six unique filters were produced; each was composed of either different sets of four descriptors or different ranges of the same set of descriptors. The best three filters are described in Table I. Their efficiencies in terms of MCC are relatively very high. Filter number 1, shown in Table I, has a MCC of 0.879, and nearly 94% of the antibacterial drugs (true positives) were successfully identified using this four-descriptor-based filter, while less than 6% of the natural products database was 'misclassified' (passed the filter as positives, but are yet unproven).

The content of the thirty-six filters was investigated; Table II shows the number of appearances of the most dominant descriptors. The third column shows how many times each descriptor actually appeared in the set of best filters, vs. random distribution. Fig. 5 was built using WORDLE module; it shows the frequency of dominant descriptors in a graphical way. The most dominant descriptors can be valued more highly than the less dominant descriptors for differentiating between antibacterial chemicals and inactive ones.

Fig. 6 describes the antibacterial activity-indexing model, showing changes in percentage of true positives, true negatives and Matthews' correlation coefficient (MCC) connected with discriminative efficiencies along with the index values. The percentages of true/false positives (left *x*-axis) and the MCCs (right *y*-axis) are plotted against the molecular bioactivity index (MBI threshold, *x*-axis). Figs. 7 and 8 show the

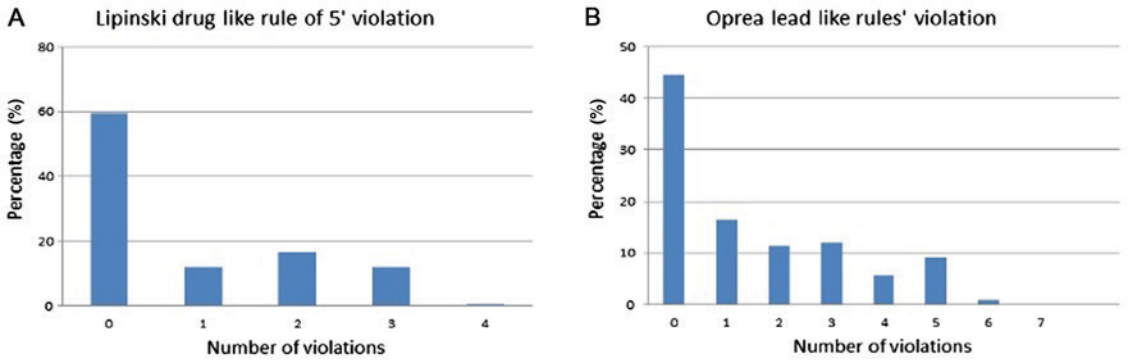

Figure 3. Distribution of the antibacterial drugs indexed by the number of violations of (A) Lipinski's rule of 5 for oral drug-likeness and (B) Oprea's rule for lead-likeness.

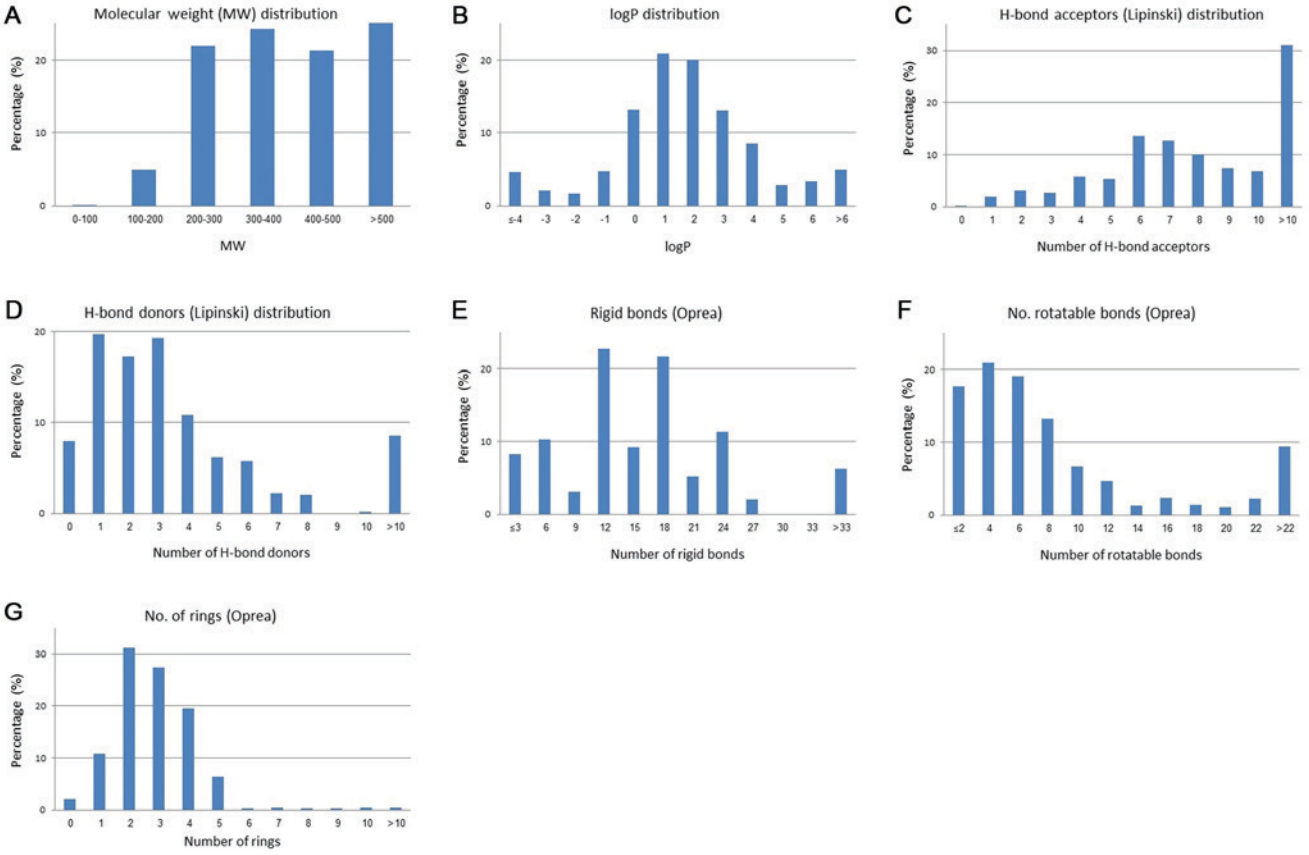

Figure 4. Distribution of the physicochemical properties of antibacterial drugs. (A) Molecular weight distribution, (B) logP values, (C) number of H-bond acceptors [lip_acc], (D) number of H-bond donors [lip_don], (E) number of rigid bonds, (F) number of rotatable bonds and (G) the number of aromatic atoms.

enrichment plot and the receiver-operating characteristic (ROC) plot of our antibacterial activity-indexing model. The enrichment plot (Fig. 7) shows how many times antibacterial drug candidates can be detected if natural products are ranked according to the ISE-based prediction model rather than random selection.

If we pick molecules with an MBI above 10.0, our predictive model and the perfect model overlay to large extent. Therefore, it seems that the indexing model is highly accurate and bears high prioritization power. With the use of this antibacterial activity-indexing model and a mixed set of active and inactive chemicals (with a ratio of 1:1,000), 72% of the antibacterial drugs were detected in the top 1% of the screened molecules, compared to 100% in the perfect model and 1% in the random model, yielding an enrichment factor of 72. The ISE-based model and the perfect model overlay to some extent in the range of MBI above 7.0. The area under the curve (AUC) attained was 0.957, which indicates that the model is excellent and highly efficient in distinguishing antibacterial drugs from inactive natural products. The natural products database, composed of 2,892 phytochemicals, was virtually screened using this filter-based activity-indexing model. The MBI scores, as shown in Fig. 6, range from-3.0 (the lowest score) to 87.0 (the highest score). Figs. 9 and 10 disclose 10 natural products that scored high as potential antibacterial drug candidates (with MBI scores above 10.0). When choosing an MBI threshold score of 10.0,

Table I. Detailed information regarding the efficiencies and ranges of descriptors for the best 3 filters out of the 36 used to construct the antibacterial indexing model.

A, Parameters for filter efficiency

Variable	Filter 1	Filter 2	Filter 3
MCC	0.879	0.809	0.782
TP (%)	93.79	81.85	79.8
TN (%)	94.09	98.03	97.2

B, Descriptors, (range)

1	BCUT_SMR_3, (0-3.33)	PEOE_PC+, (0-29.795)	PEOE_PC+, 95 (0-29.795)
2	logS, (-19.42-2.64)	a_nN, (2- 32)	PEOE_VSA_NEG, (0-1,045.34)
3	a_nN, (1-32)	Q_VSA_FNEG, (0-0.808)	a_ICM, (1.55-2.33)
4	GCUT_PEOE_3, (0-3.754)	SMR_SVA0, (0-658.98)	PEOE_VSA_FNEG, (0 -0.834)

The efficiency of the filters, in terms of their MCCs, may be very close, however, they differ in their true positive and negative percentages. In addition, the filters could be composed of different sets and/or ranges of descriptors. The descriptors and their methods of calculation can be found at www.chemcomp.com/journal/descr.htm. MCCs, Matthews correlation coefficients; TP, true positive; TN, true negative.

Table II. Number of appearances of the most dominant descriptors within the set of 36 filters that were utilized in the construction of the antibacterial model.

Descriptor name	Redundancy	Redundant more times than random
GCUT_SLOGP_0	22	36.7
a_ICM	11	18.4
PEOE_VSA+4	7	11.7
SMR_VSA1	7	11.7
logS	6	10.0
Nmol	6	10.0
lip_druglike	5	8.3
chi1_C	4	6.7
GCUT_PEOE_0	4	6.7
opr-leadlike	4	6.7
Q_VSA_FPOS	4	6.7
SMR_VSA3	4	6.7

Figure 5. A schematic drawn using WORDLE module software, which graphically illustrates the appearance of the descriptors in the 36 filters that were used for the construction of the antibacterial model.

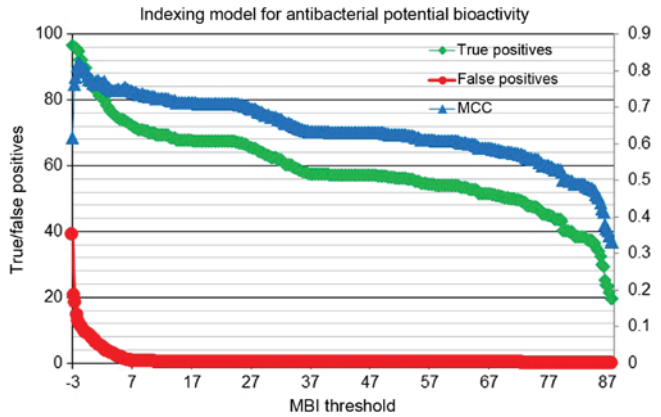

Figure 6. Antibacterial indexing model. The percentages of true/false positives (left y-axis) and the MCCs (right y-axis) plotted against the MBI values (x-axis). MCCs, Matthews correlation coefficients; MBI, molecular bioactivity index.

the ratio of TP: FP is 168:1. A search on PubMed revealed that two of the highly indexed phytochemicals (caffeine and ricinine) have already been tested experimentally and confirmed as antibacterial agents (35). Caffeine has been reported to exert physiological effects on various organisms at μM concentrations and to act as an antimicrobial agent (36,37). Ricinine had high nematocidal activity (38) and notable activity against ants (39). The other eight phytochemicals await evaluation in the wet lab to ascertain their potential antibacterial activity. It is worth mentioning that one of the volatile isolates from *Cardaria draba (L.) Desv.* that contained glucosinalbin has shown a wide range of growth inhibition activity against both Gram-positive and Gram-negative bacteria (40). It is worth testing to establish whether the phytochemical glucosinalbin is one of the main contributors to the extract's antibacterial activity. The chemical structures of caffeine, ricinine, and glucosinalbin are shown in Fig. 9. Fig. 10 displays the chemical structures of the other seven phytochemicals that scored high as potential antibacterial drug candidates with our model and await validation in the wet lab.

The current study provides vital insights into the discriminative properties of antibacterial natural products and this

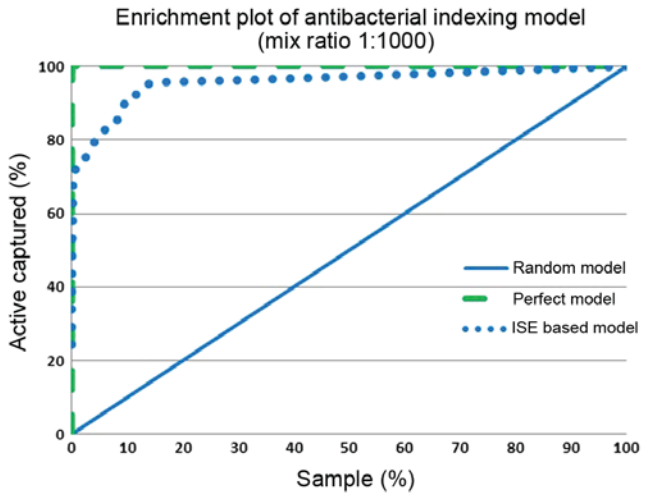

Figure 7. Enrichment plot of the antibacterial activity-indexing model. ISE, *iterative stochastic elimination*.

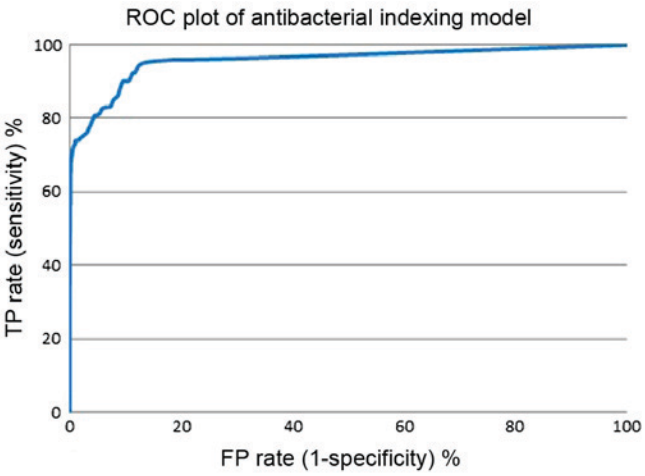

Figure 8. A ROC curve presenting the performance of the antibacterial activity-indexing model. ROC, receiver operating characteristic; TP, true positive; FP, false positive.

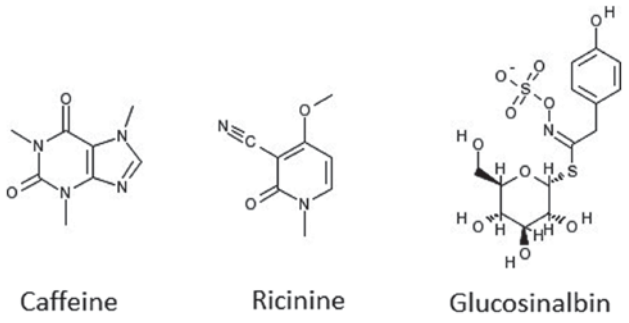

Figure 9. Chemical structures of caffeine, ricinine, and glucosinalbin.

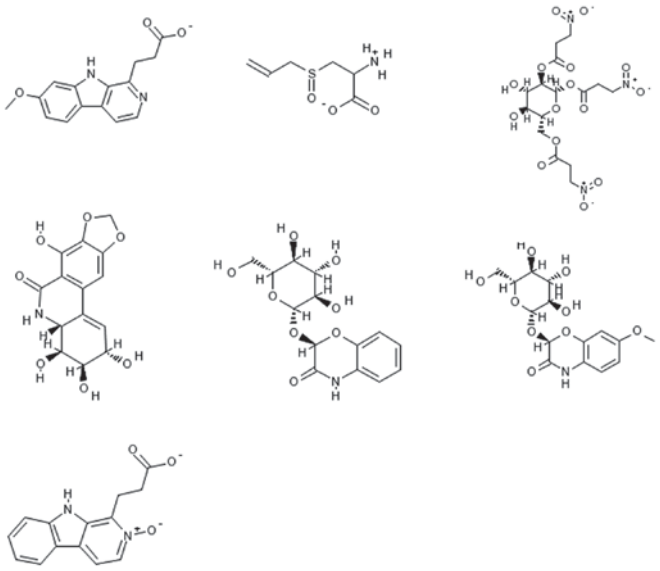

Figure 10. Remaining structures of the 7 natural products that scored highly as potential antibacterial drug candidates with the novel, *iterative stochastic elimination*-based, antibacterial activity-indexing model. These await validation in wet lab experiments.

information might be supportive to medicinal chemists in their search for novel natural antibacterial products. As well, we think the one of the aims behind publication of such theoretical work is to recruit experimental groups that are not in contact with us to test the disclosed molecules/natural products. This paper should be cited following their antibacterial activity evaluation.

It is worth to note that application of structural-based approaches in drug discovery fails to deliver better results than application of ligand-based approaches due to low efficient scoring functions and high number of false positive. The number of false positives is very high, mainly in virtual high-throughput screening, and we are waiting for development of novel scoring functions that capable to reduce the number of false positives. From previous experience in other projects, we have seen that structural-based approaches, which uses docking, could be helpful for re-ranking highly scored ligands that are output from ligand-based approaches (26). Successful story was published last year describing the utility of the ISE algorithm and physicochemical properties in discovery of novel ligands (19). As well, these days we have submitted a manuscript under the title 'Accelerating Drug Discovery Process by the Iterative Stochastic Elimination Algorithm: Discovering Novel Selective Agonists of PPAR-δ'. In this manuscript, we describe the discovery of novel molecular hits and leads for PPAR-δ by applying our combinatorial optimizing algorithm, ISE.

Using the ISE algorithm, we identified 36 unique filters that enabled us to construct a highly discriminative and robust model tailored to index natural products for their antibacterial bioactivity. For modeling purposes, we utilized a set of 628 antibacterial drugs, representing the active domain, and 2,892 natural products, representing the inactive domain. The area attained under the curve (AUC) was 0.957, indicating a highly discriminative and robust model. In this paper, we disclose ten natural products that scored high as antibacterial drug candidates with the proposed indexing model. A search on PubMed revealed that two phytochemicals (caffeine and ricinine) out of the ten highly indexed molecules have already been tested experimentally and confirmed as antibacterial agents. The other eight phytochemicals await experimental evaluation. Due to its high efficiency and rapidity, this model might be used to virtually screen large chemical databases and to index natural products for potential antibacterial bioactivity.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

AR was involved in study conception and curation, formal analysis, funding acquisition, the methodology, study supervision, and reviewing and editing the manuscript. MM contributed to the formal analysis, performed the investigations, validated the study and assisted with the original draft of the manuscript. MR performed the investigation and validation. AA and ZA contributed to the formal analysis and completed the original draft of the manuscript. MM, MR, AA, ZA and AR gave final approval for the version to be published.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interest.

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