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Comparative analysis of retrosynthesis applications for predicting of pathways for plant Secondary metabolite production

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ABSTRACT

Secondary metabolites (SMs), organic compounds synthesized by plants, play crucial roles in their own physiology and within their ecological niches, and have extensive usages in industries like pharmaceuticals, cosmetics, and food. Due to the complex nature of these metabolites, utilizing microorganisms has been proposed for their efficient and cost-effective production. In addressing challenges in SM synthesis, retrosynthesis has become essential. Applications such as RetroPath2.0, BioNavi-NP, and RetroBioCat have been developed to predict and design biosynthetic pathways, supporting synthetic biology and metabolic engineering efforts. These applications employ different methodologies to enhance the synthesis of target compounds, yet often face limitations in user-friendliness, functionality, and adaptability. This study evaluates the potential of RetroPath2.0, RetroBioCat, and BioNavi-NP in predicting the production pathways of 11 alkaloids, a class of plant secondary metabolites (PSMs). Through comparative analysis, the efficacy of these applications in proposing alternative production strategies was assessed. Findings highlighted RetroPath2.0's capability in outlining precise production pathways in host organisms, despite some restrictions in its broader practicability. The study also demonstrated the production pathways of dimethyltryptamine, nicotine, and higenamine in non-plant hosts, illustrating the practical uses of these applications.

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Secondary metabolites; retrosynthesis applications; synthetic biology; metabolic engineering; alkaloid biosynthesis

Introduction

Secondary metabolites (SMs), which are organic molecules synthesized by plants, have a wide range of effects on plants themselves and other living organisms in their environment and are used in many industrial applications, especially in pharmaceuticals, cosmetics, and food [1,2]. The extraction of these SMs, which are produced in diverse complex chemical compositions depending on stress conditions, is accomplished by following various extraction procedures from plants such as soxhlet extraction, microwave- or ultrasound-assisted extraction and other methods using organic solvents [3,4]. While many methods exist for extracting plant secondary metabolites (PSMs), they face the challenge of separating chemically similar compounds, leading to complex processes and increased costs [4,5]; to address this issue, using microorganisms has been proposed as a more efficient and cost-effective alternative for producing SMs for diverse applications [5,6]. Although higher yields have been reported to be achieved for the production of some PSMs, low and unstable production efficiency for some SMs is the most critical issue [6,7].

Alkaloids, a large family of SMs, have gained significant attention due to their diverse importance across various fields. Current studies clearly highlight their potential as medical therapeutics, agricultural biopesticides, or new approaches in food technologies [8–10]. Due to their good properties, alkaloids also take their places in metabolic and genetic engineering studies to improve their synthesis efficiencies [11].

Understanding the synthesis pathways of PSMs, especially alkaloids in this study, by alternative organisms is crucial yet challenging due to the complex mechanisms involved. Integrating experimental methods with insights from known synthesis pathways across species offers promising approaches to address this challenge [12]. One of the most prominent methods

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developed to fill in the missing gaps in the production of SMs is retrosynthesis, which provides a reverse prediction of the synthesis pathway of a target molecule by breaking it down into simpler fragments that can produce the molecule chemically or biologically [13]. RetroPath2.0, BioNavi-NP, and RetroBioCat are computational applications that predict and design biosynthetic pathways for synthetic biology and metabolic engineering [14–16]. RetroPath2.0 focuses on designing heterologous pathways, BioNavi-NP serves as a navigation system for natural products, and RetroBioCat automates the design of enzyme-catalyzed steps in synthetic pathways, each application employing unique approaches to facilitate the synthesis of target compounds. Although retrosynthesis applications classified as chemical and biological are available as web servers or standalone software, most of them are not sufficient in terms of ease of use, capabilities, and flexibility [17–19].

In this study, 11 alkaloids were selected in plant SMs and their production pathways were predicted through three retrosynthesis applications: RetroPath2.0, RetroBioCat, and Bio-Navi-NP. Among the alkaloids, all retrosynthesis applications gave comparable results for three alkaloids: Dimethyltryptamine (DMT), a terpenoid indole alkaloid produced by plants such as *Mimosa tenuiflora*, is recognized for its potential in neurorehabilitation, offering benefits such as promoting neural connections and treating brain injuries [20,21]. Higenamine (HG, a.k.a. norcoclaurine), a benzylisoquinoline alkaloid found in species like *Nelumbo nucifera* and *Nandina domestica*, has diverse pharmacological properties, including anti-inflammatory and cardioprotective effects, and is banned by the World Anti-Doping Agency due to its use in sports supplements [20,22,23]. Nicotine (Nic), a pyridine alkaloid primarily produced by *Nicotiana* species, is notable for its neuroprotective effects, particularly in relation to neurodegenerative diseases such as Parkinson's [24,25]. We demonstrated the production pathways of DMT, HG, and Nic in non-plant organisms. Our findings indicated that RetroPath2.0 was especially effective in predicting accurate pathways in non-plant hosts, although its practicality is limited for certain metabolites.

Materials and methods

Selection of SMs and data acquisition

In this study, 11 plant alkaloids (i.e. ryanodine, dimethyltryptamine, physostigmine, reserpine, strychnine, bicuculline, d-tubocurarine, higenamine (nor-coclaurine), mescalin, nicotine, and lobeline) were subjected as SMs. All chemical structure identifiers (InChI ids) were collected from PubChem [26].

Retrobiosynthesis analysis

This study aims to predict the metabolic capability for producing targeted alkaloids using three computational applications: RetroPath2.0, RetroBioCat, and BioNavi-NP.

Retrosynthesis analysis with RetroPath2.0 was conducted using the **Galaxy-SynBioCAD** platform (galaxy-synbiocad.org) [27,28]. However, RetroBioCat and BioNavi-NP were used *via* their own web interfaces.

In Galaxy-SynBioCAD Retrosynthesis workflow, RRules Parser (Galaxy Version 2.4.6), Pick SBML Model (Galaxy Version 0.0.1), Sink from SBML (Galaxy Version 5.12.1), RetroPath2.0 (Galaxy Version 2.3.0), RP2paths (Galaxy Version 1.5.0), Complete Reactions (Galaxy Version 5.12.2) were used. As the analysis parameters of the retrosynthesis workflow, all possible diameters of the reaction rules were selected. In *Target to produce* step, InChI of the SM of interest was given, for each analysis. In *Pick SBML Model* step, strain as the host vessel for the production (chassis organism) was selected correspondingly. In the RetroPath2.0 step, the maximal pathway length was selected as 10, to include all possible pathways with the highest scores. All other options were kept as their default values.

In Galaxy-SynBioCAD Pathway Analysis workflow, the inputs were retrosynthesis workflow output (previous step) and Biomass reaction that was extracted from *E.coli* iML1515 genome-scale metabolic model. Of note, the iML1515 metabolic model of *E. coli* was used because it offers comprehensive metabolic coverage, a well-characterized gene set, strong predictive power, extensive validation, flexibility for genetic modifications, and compatibility with a variety of tools [29]. The subsequent modules were run with default values, i.e. Flux balance analysis, Thermo, Score Pathway, and Rank Pathways (Galaxy Version 5.12.1). For visualizations, *Visualize pathways* tool (Galaxy Version 5.10.0) was used.

The Retrosynthesis planning tool RetroBioCat (retrobiocat.com, Version: v2023.11.30) [16] was used to analyze 11 plant alkaloids using both its Pathway Explorer and Network Explorer 2 beta approaches. In Pathway Explorer, SMILES strings were analyzed with a maximum of 5 steps and metabolites as source molecules, while Network Explorer evaluated all possible reactions and substrates. The top 3 pathway routes for each alkaloid were collected as JSON files based on their scores.

BioNavi-NP (biopathnavi.qmclab.com), a deep learning-based retrosynthesis software, was employed to analyze plant alkaloids using their InChI strings [15]. The analysis utilized core and extended libraries for building blocks, with other options set to default using quick settings. Pathways were ranked by decreasing

total costs and collected as txt files, with lower costs indicating higher reaction probability.

Results

Comparative evaluation of retrosynthesis applications via eleven alkaloids

The evaluation of RetroBioCat, BioNavi-NP, and RetroPath2.0 for predicting alkaloid production pathways revealed varying capabilities and accuracies. RetroBioCat and BioNavi-NP successfully generated results for all eleven alkaloids studied, while RetroPath2.0 provided predictions for only three: dimethyltryptamine (DMT), higenamine (HG), and nicotine (Nic) (Table 1). Despite its limited scope, RetroPath2.0 demonstrated the highest consistency with published metabolic pathways, particularly in predicting the DMT production pathway from tryptophan.

For the three alkaloids predicted by all applications, there were notable similarities and differences. RetroPath2.0 and RetroBioCat showed alignment in their predictions, especially in the final steps of HG production, though initial steps varied. BioNavi-NP's approach differed significantly, focusing on building block assembly rather than compound transformations. In the case of nicotine, the predictions of RetroPath2.0 match well with the literature [30,31], suggesting the potential involvement of previously undescribed intermediate reactions.

DMT

Notably, DMT exhibits the highest degree of similarity among the predicted pathways generated by each application. The DMT production pathway is described in the literature as the conversion of tryptophan to tryptamine by aromatic L-amino acid decarboxylase followed by the addition of a methyl group mediated by Indoleethylamine N-methyltransferase activity [32,33] (Figure 1a). RetroPath2.0 suggested that the DMT production pathway is initiated by the catalysis of tryptophan to tryptamine by aromatic-L-amino-acid decarboxylase. The addition of two methyl groups to this compound by amine N-methyltransferase results in the formation of DMT (Figure 1b). RetroBioCat predicted the initiation of the production pathway to be the conversion of indole to tryptophan by tryptophan synthase. On the other hand, no steps of BioNavi-NP were consistent with the pathways suggested by other applications. It predicted a mechanistic production pathway involving the joining of oxindole and deanol, followed by the removal of the extra oxygen, rather than biocatalytic activity.

HG

Higenamine (HG) has demonstrated diverse pharmacological properties, including anti-apoptotic, anti-thrombotic, anti-oxidative, anti-inflammatory, and immunomodulatory effects [22,23,34]. Found in edible and medicinal plants as well as sports supplements, HG has been banned by the World Anti-Doping Agency since 2017 [23,35]. The biosynthesis pathway of HG is described in the literature as the condensation of dopamine and 4-hydroxyphenylacetaldehyde [36], with earlier steps starting from tyrosine [37].

The biosynthetic pathway prediction for HG production varies among the three applications studied. RetroPath2.0 and RetroBioCat showed similarities in their top predictions, suggesting the formation of HG through the reaction of dopamine and 4-hydroxyphenylacetaldehyde (Figure 2a). RetroPath2.0's pathway begins with tyrosine conversion to tyramine and then to dopamine, while 4-hydroxyphenylpyruvic acid is converted to 4-hydroxyphenylacetaldehyde (Figure 2b), closely aligning with pathway information found in the literature (Figure 2a) [37]. BioNavi-NP's third most economical pathway aligns closest with these predictions, proposing HG biosynthesis through dopamine and 4-hydroxyphenylacetaldehyde intermediates, with dopamine derived from levodopa and

Table 1. The result status of the applications for each alkaloid.

Alkaloid	Canonical SMILES	RetroPath2.0	BioNavi-NP	RetroBioCat
Bicuculline	CN1CCC2=CC3=C(C=C2C1C4C5=C(C6=C(C=C5)OCO6)C(=O)O4)OCO3	×	✓	✓
D-tubocurarine	CN1CCC2=CC(=C3C=C2C1CC4=CC=C(C=C4)OC5=C6C(CC7=CC(=C(C=C7)O)O3)[N+](CCC6=CC(=C5O)OC)(C)C)OC	×	✓	✓
Dimethyltryptamine	CN(C)CCC1=CNC2=CC=CC=C21	✓	✓	✓
Higenamine	C1CNC(C2=CC(=C(C=C21)O)O)CC3=CC=C(C=C3)O	✓	✓	✓
Lobeline	CN1C(CCCC1CC(=O)C2=CC=CC=C2)CC(C3=CC=CC=C3)O	×	✓	✓
Mescaline	COC1=CC(=CC(=C1OC)OC)CCN	×	✓	✓
Nicotine	CN1CCCC1C2=CN=CC=C2	✓	✓	✓
Physostigmine	CC12CCN(C1N(C3=C2C=C(C=C3)OC(=O)NC)C)C	×	✓	✓
Reserpine	COC1C(CC2CN3CCC4=C(C3CC2C1C(=O)OC)NC5=C4C=CC(=C5)OC)OC(=O)C6=CC(=C(C(=C6)OC)OC)OC	×	✓	✓
Ryanodine	CC1CCC2(C3(CC4(C5(C(C(C3(C5(C2(C1O)O4)O)O)OC(=O)C6=CC=CN6)(C(C)C)O)C)O)C)O	×	✓	✓
Strychnine	C1CN2CC3=CCOC4CC(=O)N5C6C4C3CC2C61C7=CC=CC=C75	×	✓	✓

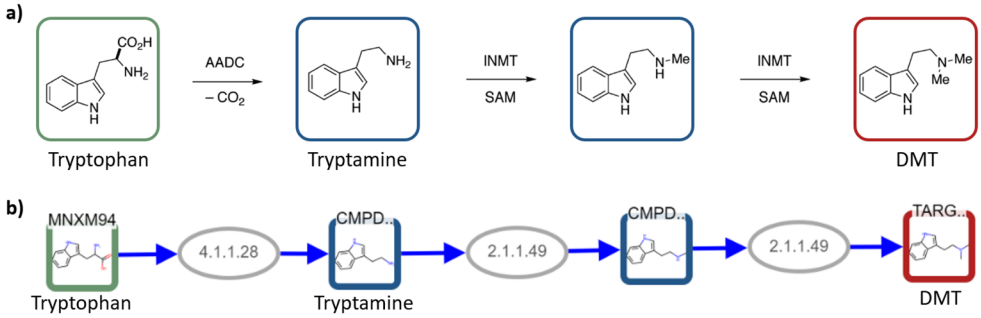

Figure 1. Biosynthesis pathway of dimethyltryptamine. (a) According to the literature. (b) The RetroPath2.0 pathway scope prediction of dimethyltryptamine visualized by the scope viewer tool. AADC: Aromatic L-amino acid decarboxylase. INMT: Indolethylamine N-methyltransferase. SAM: S-adenosylmethionine, serving as the methyl donor. 4.1.1.28: aromatic-L-amino-acid decarboxylase. 2.1.1.49: amine N-methyltransferase.

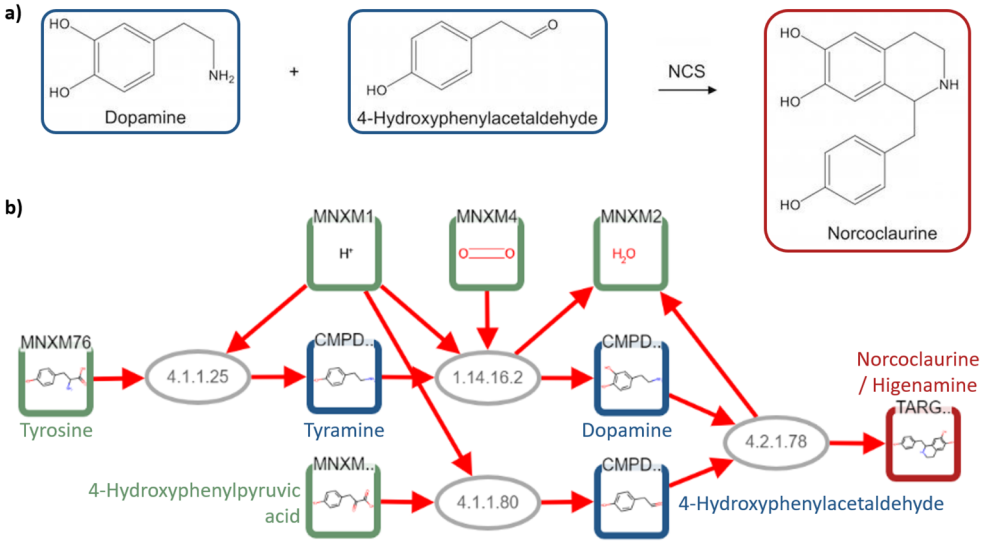

Figure 2. Biosynthesis pathway of higenamine. (a) According to the literature. (b) Pathway prediction of higenamine, visualized by the online Galaxy-SynBioCAD platform of RetroPath2.0. 4.1.1.25 – Tyrosine decarboxylase. 1.14.16.2 – Tyrosine 3-monooxygenase. 4.1.1.80 – 4-hydroxyphenylpyruvate decarboxylase. 4.2.1.78 – (S)-norcoclaurine synthase.

4-hydroxyacetaldehyde from tyrosine. These predictions provide valuable insights into potential HG biosynthetic routes, particularly in the context of the *E. coli* iML1515 model, where dopamine and 4-hydroxyphenylacetaldehyde are not naturally occurring metabolites.

Nic

While the nicotine biosynthetic pathway is not yet fully elucidated, several key steps have been characterized, and putative precursor molecules and intermediates proximal to the final step of nicotine formation have been identified and proposed. However, the final reaction that leads to the formation of the ring structure of nicotine and the enzymes responsible for it are not known [30,31] (Figure 3a).

RetroPath2.0 predicted the unknown steps in the nicotine biosynthesis pathway, starting from the precursors putrescine and nicotinic acid. According to the prediction, The transformation from putrescine continues with the formation of N-methylputrescine, catalyzed by the enzyme putrescine N-methyltransferase (EC 2.1.1.53). N-methylputrescine is then converted to N-methylpyrrolinium by the action of primary-amine oxidase (EC 1.4.3.21). On a separate branch, nicotinic acid is transformed into dihydronicotinic acid through an oxidoreduction reaction, followed by decarboxylation to form dihydropyridine, catalyzed by the enzyme 3,6-dihydronicotinate decarboxylase. The N-methylpyrrolinium and dihydropyridine intermediates are then combined by the enzyme 3,6-dihydroxynicotinate synthase to form 3,6-dihydroxynicotine [38]. Finally, 3,6-dihydroxynicotine is converted to nicotine by an oxidoreductase enzyme acting on the CH-OH group,

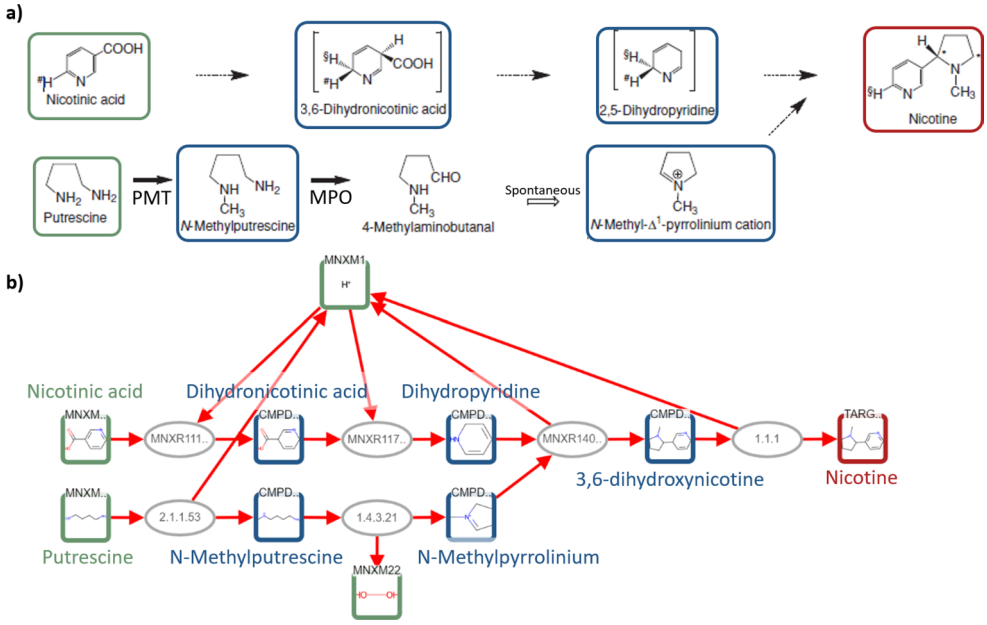

Figure 3. Nicotine biosynthesis pathway. a) According to the literature. PMT: putrescine N-methyltransferase. MPO: N-methylputrescine oxidase. Dashed arrows: undefined steps. b) Nicotine pathway prediction visualized by the online Galaxy-SynBioCAD platform of RetroPath2.0. 2.1.1.53: Putrescine N-methyltransferase. 1.4.3.21: Primary-amine oxidase. MNXR111971 (deprecated into MNXR150258 and MNXR150259; enzyme is not known): an oxidoreduction reaction. MNXR117885: 3,6-dihydronicotinate decarboxylase (EC not known). MNXR140292 (deprecated into MNXR117886; 1.1.1.–): 3,6-dihydroxynicotinate synthase.

using NAD+ or NADP+ as the electron acceptor (EC 1.1.1.-) [39] (Figure 3b). The predictions made by RetroPath2.0 provide valuable insights into the nicotine biosynthesis pathway, filling in the gaps left by previous studies [30,31].

Discussion

RetroPath2.0, BioNavi-NP, and RetroBioCat are computational tools designed to advance synthetic biology and metabolic engineering by predicting and designing biosynthetic pathways [14–16,40]. RetroPath2.0 focuses on creating heterologous pathways for the production of target compounds, using an extended reaction rule environment that allows for broad application across various molecular families [14]. BioNavi-NP employs deep learning algorithms to join blocks and estimate the least costly compounds, acting as a navigation system for exploring biosynthetic pathways and gene clusters involved in natural product synthesis [15]. RetroBioCat, on the other hand, is tailored for industrial production and emphasizes biocatalytic cascades, automating enzyme-catalyzed steps within synthetic pathways [16]. Unlike RetroPath2.0 and BioNavi-NP, it leverages both known enzyme activities and machine learning predictions to propose efficient biotransformations without requiring a living host organism.

Current research in pathway prediction primarily focuses on industrial production pathways, with less attention given to natural biosynthetic routes. A novel approach integrates synthetic and enzymatic prediction methods with neural networks, enhancing the accuracy and efficiency of pathway predictions by leveraging traditional analysis and machine learning techniques [41]. It discusses case studies comparing enzymatic, synthetic, and hybrid production routes, concluding that hybrid methods are optimal. The research utilizes the BKMS-react database [42], RetroPath2.0, and RetroRules for pathway predictions [40], while efforts are increasingly aimed at reducing costs of precursor metabolites in high-value compound synthesis [43], particularly in SM production, which is largely achieved through microorganism engineering [44]. However, progress is hindered by limited plant metabolome data and a lack of experimental validation. For example, tools like DetSpace [45] and Galaxy-SynBioCAD [28] contribute to pathway prediction by using diverse organism models, although DetSpace's requirement for predefined target molecules limits its scope.

We analyzed the utilization of three computational applications—RetroPath2.0, BioNavi-NP, and RetroBioCat—to investigate the retrosynthetic pathways of eleven alkaloids, achieving successful analyses for Dimethyltryptamine, Higenamine, and Nicotine. However, a critical limitation is

noted due to the incomplete knowledge regarding the biosynthetic pathways of five specific alkaloids: Bicuculline, Ryanodine, D-tubocurarine, Lobeline, and Mescaline. RetroPath2.0 yielded the best results, providing similar steps to the biosynthesis pathways in the literature; however, it does not work for all cases, and requires rule files to be prepared beforehand. It is rule-based and strict to produce pathways out of its reaction rule set. RetroBioCat is a promising alternative and provides pathways similar to actual pathways. Also, it operates online, making it more convenient in that regard. As for BioNavi-NP, it is still not quite ready for use with this purpose, as it followed pathways that are not present in the literature. When RetroPath2.0 is ineffective for the three alkaloids (Physostigmine, Reserpine, and Strychnine), whose biosynthetic pathways are documented in the literature, both RetroBioCat and BioNavi-NP fail to approximate the literature pathways and cannot reach a consensus. Hence, an effective retrobiosynthesis strategy may involve the initial implementation of RetroPath2.0, followed by RetroBioCat, to elucidate potential synthetic pathways. The findings of this study on a small set of secondary metabolites indicate that further research is required to improve the consistency of retrosynthetic analyses and to elucidate the biosynthetic origins of metabolites. Future research will be necessary to improve the understanding of metabolic networks and to enhance the computational methods used in alkaloid pathway analysis.

Conclusion

In conclusion, RetroPath2.0 stands out as the leading tool for predicting metabolic pathways, thanks to its incorporation of host metabolism and validation of pathway feasibility. Although it lacks experimental validation, it provides valuable insights for researchers investigating secondary metabolite pathways across different organisms. However, its dependence on precursor molecules and often incomplete metabolic models for plants poses a significant challenge. Enhancing its query capabilities to include intermediate compounds and enzymes could significantly improve the tool's reliability and breadth. A complementary approach to RetroPath2.0 could involve using it initially, followed by RetroBioCat to investigate potential synthetic pathways. The findings highlight the need for further research to improve the efficiency of retrosynthetic analyses and clarify the biosynthetic origins of complex metabolites, emphasizing that a deeper understanding of metabolic networks and enhanced computational methods are crucial for advancing alkaloid pathway analysis and optimizing synthetic biology applications.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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Data availability statement

The authors confirm that the data supporting the findings of this study are available within the article.

References

[1] Teoh ES. Secondary metabolites of plants. In: Medicinal Orchids of Asia, 1st ed. Cham, Switzerland: Springer; 2016. p. 59–73. doi:10.1007/978-3-319-24274-3_5.
[2] Guerriero G, Berni R, Muñoz-Sanchez JA, et al. Production of plant secondary metabolites: examples, tips and suggestions for biotechnologists. Genes. 2018;9(6):309. doi:10.3390/genes9060309.
[3] Jones WP, Kinghorn AD. Extraction of plant secondary metabolites. New Jersey: Humana Press; 2012. p. 341–366. doi:10.1007/978-1-61779-624-1_13.
[4] Abubakar A, Haque M. Preparation of medicinal plants: basic extraction and fractionation procedures for experimental purposes. J Pharm Bioallied Sci. 2020;12(1):1–10. doi:10.4103/jpbs.jpbs_175_19.
[5] Kallscheuer N, Classen T, Drepper T, et al. Production of plant metabolites with applications in the food industry using engineered microorganisms. Curr Opin Biotechnol. 2019;56:7–17. doi:10.1016/j.copbio.2018.07.008.
[6] Wawrosch C, Zotchev SB. Production of bioactive plant secondary metabolites through in vitro technologies—status and outlook. Appl Microbiol Biotechnol. 2021;105(18):6649–6668. doi:10.1007/s00253-021-11539-w.
[7] Méndez-Hernández JE, Rodríguez-Durán LV, Páez-Lerma JB, et al. Strategies for supplying precursors to enhance the production of secondary metabolites in solid-state fermentation. Fermentation. 2023;9(9):804. doi:10.3390/fermentation9090804.
[8] Chakravorty J. Prey predator interaction with reference to chemical cues or bioactive compounds. New Delhi, India Bright Sky Publications; 2024.49–67., pp.,
[9] Gaikwad SV, Pai SR. Ethnobotany of capsaicin-producing plants. Singapore: Springer; 2024. p. 55–112., doi:10.1007/978-981-99-7779-6_3.
[10] Kumar S, Sarpras M, Mushtaq F, et al. Capsaicin-producing plants: species diversity, distribution and botanical details. Singapore: Springer; 2024. p. 1–24. doi:10.1007/978-981-99-7779-6_1.
[11] Leonard E, Runguphan W, O'Connor S, et al. Opportunities in metabolic engineering to facilitate scalable alkaloid production. Nat Chem Biol. 2009;5(5):292–300. doi:10.1038/nchembio.160.
[12] Nair VH, Schwaller P, Laino T. Data-driven chemical reaction prediction and retrosynthesis. Chimia. 2019;73(12):997–1000. doi:10.2533/chimia.2019.997.

[13] Sun Y, Sahinidis NV. Computer-aided retrosynthetic design: fundamentals, tools, and outlook. Curr Opin Chem Eng. 2022;35:100721. doi:10.1016/j.coche.2021.100721.
[14] Delépine B, Duigou T, Carbonell P, et al. RetroPath2.0: a retrosynthesis workflow for metabolic engineers. Metab Eng. 2018;45:158–170. doi:10.1016/j.ymben.2017.12.002.
[15] Zheng S, Zeng T, Li C, et al. Deep learning driven biosynthetic pathways navigation for natural products with BioNavi-NP. Nat Commun. 2022;13(1):3342. doi:10.1038/s41467-022-30970-9.
[16] Finnigan W, Hepworth LJ, Flitsch SL, et al. RetroBioCat as a computer-aided synthesis planning tool for biocatalytic reactions and cascades. Nat Catal. 2021;4(2):98–104. doi:10.1038/s41929-020-00556-z.
[17] Campodonico MA, Andrews BA, Asenjo JA, et al. Generation of an atlas for commodity chemical production in escherichia coli and a novel pathway prediction algorithm, GEM-Path. Metab Eng. 2014;25:140–158. doi:10.1016/j.ymben.2014.07.009.
[18] Jeffryes JG, Colastani RL, Elbadawi-Sidhu M, et al. MINEs: open access databases of computationally predicted enzyme promiscuity products for untargeted metabolomics. J Cheminform. 2015;7(1):44. doi:10.1186/s13321-015-0087-1.
[19] Hadadi N, Hafner J, Shajkofci A, et al. ATLAS of biochemistry: a repository of all possible biochemical reactions for synthetic biology and metabolic engineering studies. ACS Synth Biol. 2016;5(10):1155–1166. doi:10.1021/acssynbio.6b00054.
[20] Böttger A, Vothknecht U, Bolle C, et al. Alkaloids. Cham, Switzerland: Springer; 2018. p. 179–203. doi:10.1007/978-3-319-99546-5_12.
[21] Colosimo FA, Borsellino P, Krider RI, et al. The clinical potential of dimethyltryptamine: breakthroughs into the other side of mental illness, neurodegeneration, and consciousness. Psychoactives. 2024;3(1):93–122. doi:10.3390/psychoactives3010007.
[22] Zhang N, Lian Z, Peng X, et al. Applications of higenamine in pharmacology and medicine. J Ethnopharmacol. 2017;196:242–252. doi:10.1016/j.jep.2016.12.033.
[23] Cohen PA, Travis JC, Keizers PHJ, et al. The stimulant higenamine in weight loss and sports supplements. Clin Toxicol. 2019;57(2):125–130. doi:10.1080/15563650.2018.1497171.
[24] Ruan S, Xie J, Wang L, et al. Nicotine alleviates mptp-induced nigrostriatal damage through modulation of jnk and erk signaling pathways in the mice model of parkinson's disease. Front Pharmacol. 2023;14:1088957. doi:10.3389/fphar.2023.1088957.
[25] Zhgenti N, Bibilashvili O, Burjanadze G, et al. Exploring the neuroprotective effects of nicotine against mptp-induced neuronal damage in mice: insights into antioxidant system. IJBB. 2023;11(3):27–34. doi:10.13189/ijbb.2023.110301.
[26] Kim S, Chen J, Cheng T, et al. PubChem 2019 update: improved access to chemical data. Nucleic Acids Res. 2019;47(D1):D1102–D1109. doi:10.1093/nar/gky1033.
[27] King ZA, Lu J, Dräger A, et al. BiGG models: a platform for integrating, standardizing and sharing genome-scale models. Nucleic Acids Res. 2016;44(D1):D515–522. doi:10.1093/nar/gkv1049.
[28] Hérisson J, Duigou T, Lac M, et al. The automated Galaxy-SynBioCAD pipeline for synthetic biology design and engineering. Nat Commun. 2022;13(1):5082. doi:10.1038/s41467-022-32661-x.
[29] Monk JM, Lloyd CJ, Brunk E, et al. iML1515, a knowledgebase that computes escherichia coli traits. Nat Biotechnol. 2017;35(10):904–908. doi:10.1038/nbt.3956.
[30] Shoji T, Hashimoto T. Nicotine Biosynthesis. West Sussex, United Kingdom: John Wiley and Sons; 2011. doi:10.1002/9781119991311.ch7.
[31] Zenkner FF, Margis-Pinheiro M, Cagliari A. Nicotine biosynthesis in nicotiana: a metabolic overview. Tobacco Science. 2019;56(1):1–9. doi:10.3381/18-063.
[32] Baxter C, Slaytor M. Biosynthesis and turnover of n, n-dimethyltryptamine and 5-methoxy-n, n-dimethyltryptamine in phalaris tuberosa. Phytochemistry. 1972;11(9):2767–2773. doi:10.1016/s0031-9422(00)86511-5.
[33] Cameron LP, Olson DE. Dark classics in chemical neuroscience: n, n-dimethyltryptamine (dmt). ACS Chem Neurosci. 2018;9(10):2344–2357. doi:10.1021/acschemneuro.8b00101.
[34] Bai G, Yang Y, Shi Q, et al. Identification of higenamine in radix aconitilateralis preparataas a beta2-adrenergic receptor agonist1. Acta Pharmacol Sin. 2008;29(10):1187–1194. doi:10.1111/j.1745-7254.2008.00859.x.
[35] Grucza K, Kwiatkowska D, Kowalczyk K, et al. Analysis for higenamine in urine by means of ultra-high-performance liquid chromatography–tandem mass spectrometry: interpretation of results. Drug Test Anal. 2018;10(6):1017–1024. doi:10.1002/dta.2331.
[36] Menéndez-Perdomo I, Facchini P. Benzylisoquinoline alkaloids biosynthesis in sacred lotus. Molecules. 2018;23(11):2899. doi:10.3390/molecules23112899.
[37] Beaudoin GAW, Facchini PJ. Benzylisoquinoline alkaloid biosynthesis in opium poppy. Planta. 2014;240(1):19–32. doi:10.1007/s00425-014-2056-8.
[38] Moretti S, Tran VDT, Mehl F, et al. MetaNetX/MNXref: unified namespace for metabolites and biochemical reactions in the context of metabolic models. Nucleic Acids Res. 2021;49(D1):D570–D574. doi:10.1093/nar/gkaa992.
[39] Chang A, Jeske L, Ulbrich S, et al. BRENDA, the ELIXIR core data resource in 2021: new developments and updates. Nucleic Acids Res. 2021;49(D1):D498–D508. doi:10.1093/nar/gkaa1025.
[40] Duigou T, Lac M, Carbonell P, et al. RetroRules: a database of reaction rules for engineering biology. Nucleic Acids Res. 2019;47(D1):D1229–D1235. doi:10.1093/nar/gky940.
[41] Levin I, Liu M, Voigt CA, et al. Merging enzymatic and synthetic chemistry with computational synthesis planning. Nat Commun. 2022;13(1):7747. doi:10.1038/s41467-022-35422-y.
[42] Lang M, Stelzer M, Schomburg D. BKM-react, an integrated biochemical reaction database. BMC Biochem. 2011;12(1):42. doi:10.1186/1471-2091-12-42.
[43] Gao D, Song W, Wu J, et al. Efficient production of l-homophenylalanine by enzymatic-chemical cascade catalysis. Angew Chem Int Ed. 2022;61(36):77. doi:10.1002/anie.202207077.
[44] Lv X, Zhou X, Ma J, et al. Engineered saccharomyces cerevisiae for the de novo biosynthesis of (-)-menthol. J Fungi. 2022;8(9):982. doi:10.3390/jof8090982.
[45] Martín Lázaro H, Marín Bautista R, Carbonell P. DetSpace: a web server for engineering detectable pathways for bio-based chemical production. Nucleic Acids Res. 2024;52(W1):W476–W480. doi:10.1093/nar/gkae287.