Integration of Bioinformatics and Biotechnology: A Potential Insight into Antidiabetic Drug Discovery

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Abstract

Diabetes mellitus is a complex non-communicable metabolic disorder that can lead to serious health complications such as heart disease, kidney failure, blindness, and peripheral neuropathy if not treated early. Its global prevalence is rising, demanding safer and more effective therapeutic options. Medicinal plants have long served as sources of bioactive compounds precursor to effective drugs against some longstanding diseases. However, there is variability in the phytochemical composition of plant material collected from the natural environment, especially due to environmental factors, seasonal changes, and genetic variation, which often limit research reproducibility. This review highlights integration of bioinformatics and biotechnology may address these challenges and improve drug discovery efficiency. Bioinformatics tools, especially molecular docking and ADMET prediction, enable the easy and quick identification and evaluation of plant-derived compounds targeting key diabetic proteins like insulin receptors, Glucagon-like peptide 1 (GLP-1), dipeptidyl peptidase -4 (DPP-4) inhibitors and Glucose-dependent insulinotropic polypeptide (GIP) . These computational approaches reduce time, cost, and experimental uncertainty while predicting drug-like properties prior to in vitro or in vivo validations. Complementarily, biotechnology techniques such as tissue culture ensure the consistent production of genetically stable, disease-free plant materials rich in secondary metabolites independent of seasonal or environmental variations. Analytical methods like HPLC, GC-MS, and NMR further aid in compound isolation and characterization for further research to validate the potency of the identified and isolated compounds. Integration of bioinformatics and biotechnology therefore bridges the traditional medicinal plant research and modern drug discovery, ensuring reproducibility, standardization, and biodiversity conservation for antidiabetic drug discovery

Keywords: Bioinformatics, Biotechnology, Diabetes, Medicinal Plant, Drug Discovery

Introduction

Diabetes is a complex metabolic disorder characterized by chronic hyperglycemia resulting from insufficient insulin production or ineffective insulin action (Hameed *et al*., 2015). This disorder is one of the major non-communicable diseases that contributes to the global burden of disease (Lin *et al*., 2020). According to the World Population Review, the estimated number of people living with diabetes worldwide is projected to reach 720 million by 2025. In Nigeria alone, about 3.6 million people are currently living with diabetes, along with increasing

numbers across other African countries. If not properly managed, diabetes can lead to serious health complications such as heart disease, kidney failure, blindness, and nerve damage (García *et al*., 2018). Its long-term impact also includes an increased risk of stroke, amputation, and premature death as shown in Figure 1.

Medicinal plants has been used in traditional medicine for a long term, which the therapeutic potential of many plant species. In vivo studies have validated the antidiabetic potential of some of these plants, making them promising sources for drug discovery. These plants contain bioactive compounds such as flavonoids, alkaloids, terpenoids, and saponins, which have been shown to lower blood glucose levels and support β-cell function. Flavonoids from *Moringa oleifera* showed strong antidiabetic effects (Setyani *et al*., 2025). However, a major challenge that the quantity and quality of these compounds are not consistent because of environmental conditions, plant genetics, and seasonal changes. Additionally, plants collected from natural environments may be infected with diseases that have not yet manifested, of which can be a challenge in reproducing for research purposes.

Biotechnology and bioinformatics are important tools for addressing these challenges. Tissue culture, also known as micropropagation, is a type of invitro propagation where a small part of plant tissue, known as an explant is cultured in a nutrient medium under controlled environment. One of the application of tissue culture is production of secondary metabolites which enables the production of bioactive compounds that are consistent and reproducible under stable environmental conditions (Danova, 2023) (Fig 2). It also allows the production of disease-free plant materials for extraction and further studies. Bioinformatics on the other hnd has contributed to drug discovery by using computational tools to validate plant compounds before in vivo stidies. These tools can predict how phytochemicals interact with important diabetes-related proteins such as α-glucosidase, DPP-4, and the insulin receptor, and also assess their pharmacokinetic properties, safety, and absorption. Bioinformatics helps to save time, reduce costs, and complement in vitro and in vivo studies.

Existing invivo and invitro studies on some medicinal plant has shown their antidiabetic potential, including numerous in silico analyses that predicts mechanisms of action in both type 1 and type 2 diabetes. However, few have addressed how these findings can be made reproducible beyond seasonal variations, nor have they emphasized the importance of using disease-free, genetically stable plants in drug discovery while conserving biodiversity. Therefore, this paper aims to show how bioinformatics tools and biotechnology can be combined to ensure the reproducible and consistent production of bioactive compounds for improved antidiabetic research, including animal and clinical studies.

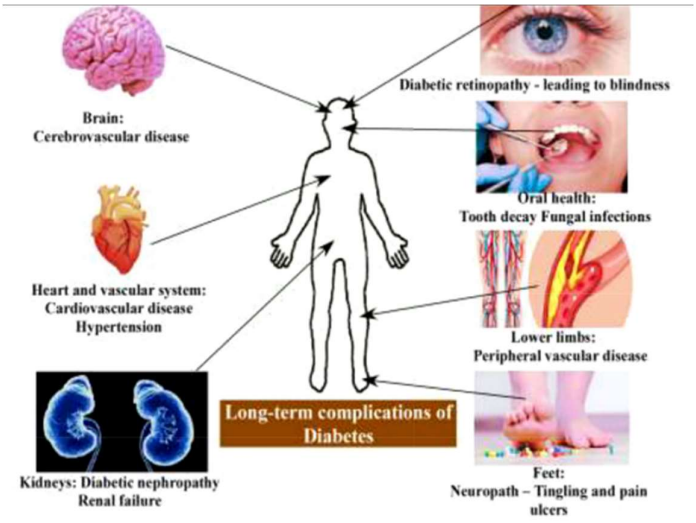

Fig 1: Longterm complications of diabetes (Lankatillake C et.al, 2019)

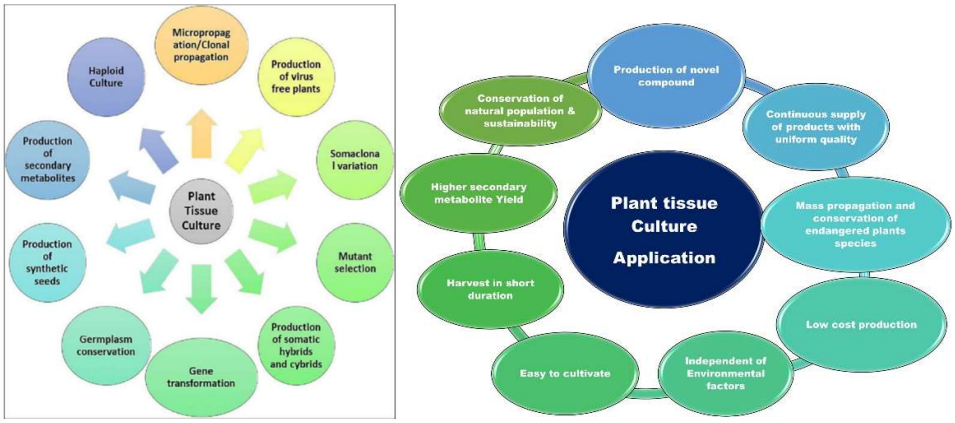

Fig 2: Applications and importance of Tissue culture

2. Bioinformatics in Drug Discovery

In silico studies is a vital part of bioinformatics used in drug development. Molecular docking is a tool that plays a key role in predicting how a small molecule interacts with a protein (Sahoo *et al*., 2022). This tools allows researchers to predict how small molecules behave within a target protein's binding site and understand the basic biochemical processes behind these interactions (Meng *et al*., 2011). Several computational tools have been created for molecular docking; according to Sahoo *et al*. (2022), the most used software are AutoDock Vina, Discovery studio, Glide, and AutoDock GOLD because of their high accuracy and reliability in scoring and prediction (Potluri *et al*., 2021).

Finding practical target proteins and ligands is the first step in carrying out docking. Target proteins are obtained from the Protein Data Bank (PDB), while ligands can be sourced from databases such as PubChem or ChEMBL, or can be drawn using tools like ChemDraw (Aja *et*

al., 2021). Protein preparation is another important step before molecular docking and it involves obtaining the protein structure from databases or modeling tools like SWISS-MODELER. Completion of any missing atoms or residues, removal of unwanted molecules, and setting of appropriate protonation states for charge balance are done during protein preparation. Ligands to be docked should be selected based on biological activity, or potential for drug development. Charges assigning, conformers generations, and optimization of their geometry are ways to prepare ligands (Chaudhary, 2016). Active site prediction involves locating or confirming where a ligand binds to a protein using one of three methods; docking directly on the site when the binding site is known, blind docking when the binding site is unknown, or standard-based docking using a known ligand as a reference (Aja *et al*., 2021). During the docking, the ligand is docked against the protein, docking score, binding affinity are part of resulting interactions which are analyzed. Post-docking analysis is the evaluation of docked ligands to identify the most promising candidates by calculating their binding affinities, ranking them based on interaction energy, and analyzing key interactions such as hydrogen bonds, hydrophobic, and electrostatic forces, which explains clearly the ligand mechanisms and also guide further structural optimization (Pinzi, 2019). Bioinformatics also helps in the to predict Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADMET) properties of small molecules,which reveals compounds with unfavorable characteristics at the early stage in the drug discovery process (Das *et al*., 2020). The general docking process is represented in a flow as shown in Fig. 3.

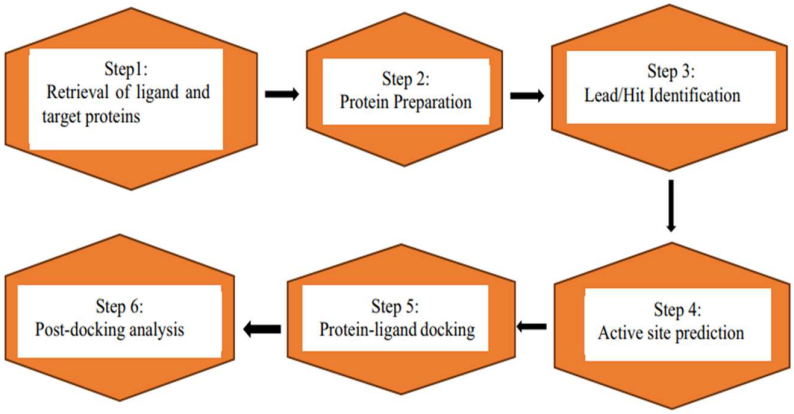

Fig 3: Flowchart of the Molecular Docking Process (Agu *et al*., 2023)

3 Medicinal Plants a Source of Antidiabetic Drug Disccovery

Moringa oleifera, *Withania somnifera*, and *Bryophyllum pinnatum* and other plants have long been used in the management of diabetes traditionally, and modern studies have validated their therapeutic potential. Phytochemicals such as flavonoids, alkaloids, terpenoids, and saponins have displayed hypoglycemic effect as shown in details in Fig 4 by improving insulin sensitivity, promoting glucose uptake, and inhibiting carbohydrate-hydrolyzing enzymes (Patel & Mishra, 2021). Quercetin isolated from *Moringa oleifera* has been shown to improve insulin sensitivity and reduce hyperglycemia in diabetic models (Ezzat *et al*.,

2020). Withanolides from *Withania somnifera* exhibit potent glucose-lowering and antioxidant effects (Sangwan *et al*., 2021). Similarly, bufadienolides derived from *Bryophyllum pinnatum* have been reported to enhance glucose uptake in vitro, highlighting their pharmacological relevance (Akinmoladun *et al*., 2022). These studies collectively confirm and validate the efficacy of traditionally used antidiabetic plants and emphasize the need to isolate and characterize the bioactive compounds responsible for their therapeutic potency for further validation.

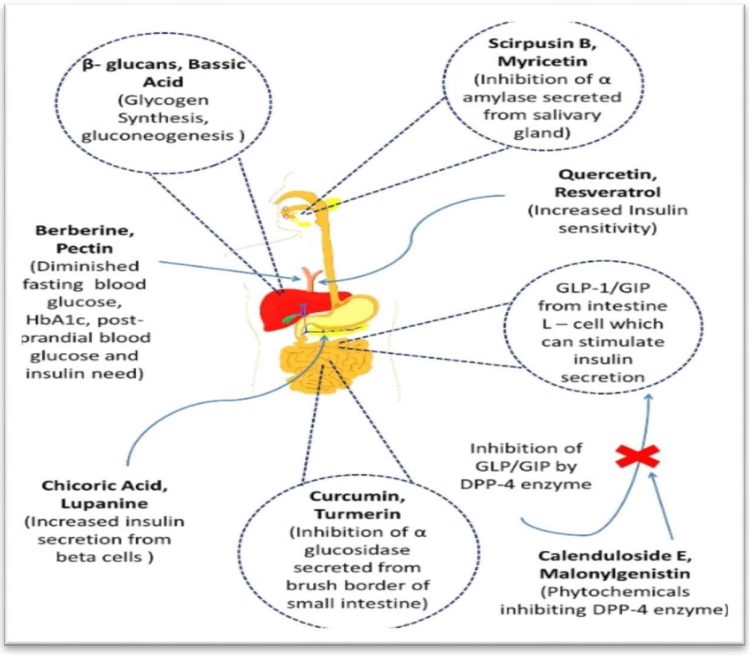

Fig 4: The mechanisms of action of several prospective bioactive secondary metabolites (phytochemicals) obtained from different medicinal plants. (Alam.S et., al 2022)

3.1 Biotechnology in Antidiabetic Drug Discovery Research

Biotechnology provides good resources to identify, standardize, and validates medicinal plants with potential antidiabetic properties. Tissue culture is a technique in biotechnology which has proven to be an effective method for propagating valuable medicinal plants, with over 1,000 species reported to be successfully cloned through this technique. It is currently commonly used to study secondary metabolites in plants, most especially for therapeutic purposes. Secondary metabolites such as alkaloids, phenols, anthocyanins, flavonoids, saponins, tannins, terpenes, and coumarins have shown significant antidiabetic potential (Chauhan *et al*., 2020; Sun *et al*., 2020). Among these, polyphenols and flavonoids showed a strong antioxidant properties, enabling them to scavenge free radicals and reduce oxidative stress, which is a major factor in the management and treatment of diabetes mellitus (Akbari *et al*., 2022).

Therefore, plant tissue culture is seen as an important biotechnological tool in modern medicinal plant research. Researchers have been able to produce genetically stable, disease-free plant parts that are responsible for the production of bioactive secondary metabolites of

which are consistent independent of environmental or seasonal variations through plant tissue culture techniques such as clonal micropropagation, callus culture, hairy root culture, and protoplast culture (Mishra, 2015; Chauhan *et al*., 2020).

3.2 Tissue culture and compound isolation.

Plant tissue culture was first introduced by Haberandt (Asmita *et al*., 2023) and has been in use for years, the process involves several important steps, each requiring adequate sterilization protocol to prevent contamination and ensure the production of disease-free plants. The culture medium includes MS powder formulated by Murashige and Skoog (Bettoni *et al*., 2019), sucrose as a carbohydrate source, essential micro and macroelements, agar for solidification which serves as anchor for the plant, and appropriate growth hormones. The medium combined is the nutritional requirements necessary for plant growth and development which must have a ph of 5.8+- 0.1. Tissue culture processes does not only ensure the continuous availability of bioactive compounds throughout the year but also conserve plant species and protect biodiversity (Sehgal & Khan, 2020). The techniques have been useful in enhancing the yield of secondary metabolites such as flavonoids, alkaloids, and terpenoids many of which possess strong antidiabetic activity (Chauhan *et al*., 2020)

Plants naturally produce varieties of secondary metabolites that are not directly involved in primary metabolic processes. These compounds often accumulate in high concentrations within certain tissues or organs and can take up to 1–3% of a plant's dry weight (Morris *et al*., 2021). Secondary metabolites is as important materials in the initial stage of drug discovery and development. Therefore, the extraction and characterization of these compounds are crucial. Extraction techniques can be traditional or modern, depending on the desired yield and purity (Ozyigit *et al*., 2023).

High-performance liquid chromatography (HPLC), mass spectrometry (MS), and nuclear magnetic resonance (NMR) spectroscopy are used for isolating and characterizing bioactive phytochemicals from plant extracts. Identified compounds can then be computationally screened and docked against diabetes-related protein targets such as the insulin receptor, α-glucosidase. Also the pharmacokinetics profiles can be done before isolating the compound with potential antidiabetic activity. Promising candidates should then be validated through in vitro and in vivo studies to confirm their biological activity, safety, and efficacy..

4. Secondary Metabolite Production

One of the limitations of using plants harvested directly from the field is that the concentration of bioactive compounds varies because of genetics, environmental factors, season, and the presence of disease-causing microorganisms.Several secondary compounds, has been successfully produced using tissue culture techniques from various explant sources, the compounds are phenolics (caffeic acid, rosmarinic acid and hexoside, salvianolic acid, K, F and isomer I, II, caffeic acid derivative I and II, and methyl rosmarinate) from the leaves and shoots of *Salvia bulleyana* (Wojciechowska *et al*., 2020); iridoid glycosides (aucubin, harpagide, harpagoside) and phenylethanoid glycosides (verbascoside and isoverbascoside)

from the seeds, leaves and shoots of *Rehmannia elata* (Piatczak *et al.,* 2019); phenolic acids (Rosmarinic acid, Caffeic acid, Lithospermic acid, Chlorogenic acid, Cinnamic acid) from the leaves and shoots of *Mentha spicata* (Yousefian *et al*., 2020); flavonoids, phenylpropanoids, alkaloids, fatty acids and aromatic glycosides from callus and suspension cultures of *Carthamus tinctorius* (Liu *et al*., 2021), phenolic acids (Caffeic acid, Syringic acid, p-Coumaric acid, ferulic acid, Salicylic acid), flavonoids (rutin, Myricetin and Kaempferol) from the nodes, internodes and leaves of *Sphagneticola calendulacea* (Kundu *et al*., 2018) and phenolics, flavonoids, tannins and essential oils from nodal segments of *Artemisia arborescens* (Riahi *et al*., 2022). Plant tissue culture techniques as shown in Fig 5 is an efffective way to produce genetically stable and disease-free material for secondary metabolite production (Kumari & Sharma, 2020).

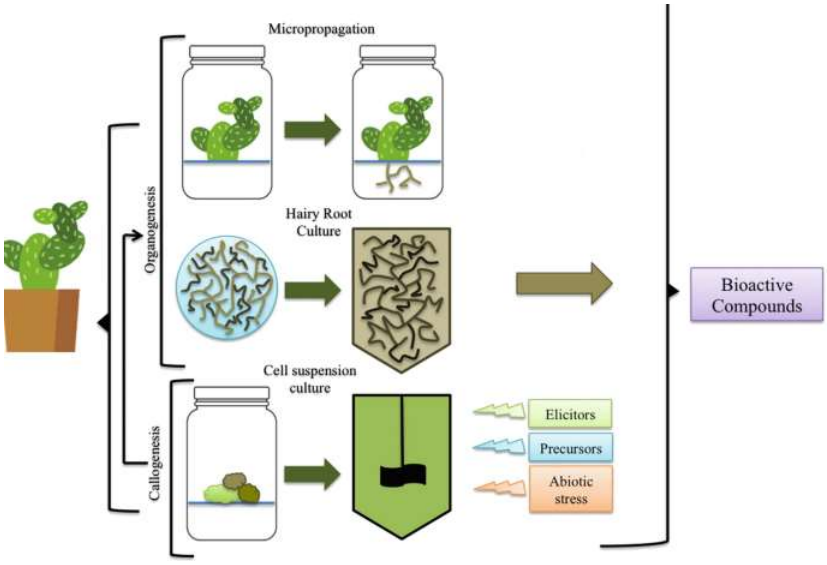

Fig 5: In vitro propagation techniques for the production of secondary metabolite

4.1 Bioinformatics in Phytomedicine Research

Recent studies in antidiabetic research has been using both bioinformatics and experimental methodology to validates plants therapeutic potientials instead of depending only on computational predictions (insilico studies) or in vivo experiments using animal models. Researchers use molecular docking to predict interactions of plant-derived compounds with antidiabetic protein targets such as α-amylase, α-glucosidase, and insulin receptors. These predictions are then validated through animal models or invitro to confirm their pharmacological activity.

According to Vo Van *et al*. (2022), they combined in silico, in vivo, and in vitro studies to validate the potential of *Merremia tridentata* (L.) to have antidiabetic potential. Apigenin cosmosiin, cynaroside, luteolin, and quercetin were identified to be present and isolated from the stem of the plant, which exhibited strong hypoglycemic effects in alloxan-induced diabetic mice. The insilico study involved docking of the flavonoids to form a complex with α-amylase, α-glucosidase, PPAR-γ and other proteins. Cynaroside and cosmosin are the compounds

showed hypoglycemic effects in many of antidiabetic targets against the proteins, and quercitrin is a potential compound for PPAR-γ activation (Vo Van *et al*., 2022). Other studies are shown in Table 1.

However, while these studies reveals the advantage of combining both computational and experimental approach, most of them used plants collected from natural habitats, of which has many limitations. They didn't address reproducibility challenges which is connected to seasonal variation, genetic stability, and standardization of bioactive metabolites, which are important factors in drug discovery. This shows the need to integrate biotechnology such as tissue culture alongside bioinformatics and pharmacological testing to ensure consistency in bioactive compound as well as reproducible research

Plant and compound extract	In Silico Tools/Protein	Experimental model (in vivo or in vitro)	Results	Reference
Allium hookeri (flavonoid)	(SUR1, K_ATP receptor	Type 2 diabetic rats	Reduced fasting blood glucose from ~317 ± 12.8 to ~99.4 ± 6.67 mg/dL; improved histology & lipid profile	Singh *et al*., 2024
Catharanthus roseus extracts (methanol/ethanol)	Molecular docking and insilico modelling	STZ-induced diabetic mice + enzyme assays	Extracts showed antidiabetic and antilipidemic effects; docking supported possible binding on target proteins	Lenh Vo Van *et al*., 2022
Chickpea (Cicer arietinum) & Barley (Hordeum vulgare) seed extracts	α-amylase and α-glucosidase	STZ-induced diabetic mice	Both seed extracts inhibited enzymes (IC□□ values better than acarbose), and improved blood glucose in vivo.	Shahzad *et al*., 2025

Table 1: Studies Combining In Silico and Experimental Validation in Antidiabetic Research

5 . Challenges and Future Perspectives

The medicinal value of plants lies in their bioactive compounds also known as secondary metabolite constituents, they contributes to some physiological results in humans (Mohiuddin 2019). However, environmental factors such as change in temperature, humidity and soil type can affect the genetic stability of plants, resulting into inconsistent phytochemical constituents (Saheed *et al*., 2021). Seasonal changes also has impact on the phytochemical composition,

especially volatile compounds being the mostly affected compounds. Research results showed that the quantity of phytochemicals changes with season, indicating that there could be a challenge of inconsistency in the plants extracts gotten directly from the field for antidiabetic research making it not to be reproducible. Additionally, plants collected may be affected by hidden diseases which is yet to manifest, which can influence the plant's phytochemicals and extracted materials.

Tissue culture techniques, as a biotechnological method, uses explants in sterile conditions and controlled media to produce disease-free plant material, independent of environmental factors, and available all year round. Genetic fidelity tests have proven that in vitro propagated plants are genetically compatible with their mother plants. Secondary metabolites are usually concentrated in specific plant parts, and tissue culture has been shown to produce plant materials with even higher levels of these compounds compared to the mother plant in natural environment. This makes it a good techniques for addressing the existing challenge thereby contributing greatly to antidiabetic research and new drug discovery.

Most of the antidiabetic research used crude plant extract to validate the efficacy of medicinal plants for certain diseases, especially diabetes. Only a few used isolated bioactive compounds responsible for such an antidiabetic effect, which is a good step closer to drug discovery. Bioinformatics tools on the other hand like absorption, distribution, metabolism, excretion, and toxicity (ADMET) predictions provide insight into the pharmacokinetic and drug-like properties of compounds, helping researchers identify the most promising compounds (Wu *et al*., 2020). Molecular docking can be used to screen for potential drug candidates and filter out compounds with unfavorable absorption, distribution, metabolism, excretion, and toxicity (ADMET) properties early in drug discovery (Agu *et al*., 2023). Isolating potent compounds and validating them in vitro or in animal models is therefore a key step toward developing new drugs for diabetes.

6. Conclusion

Diabetes mellitus remains a major global health challenge, and medicinal plants will continue to be a source of antidiabetic drug discovery due to their diverse secondary metabolites. However, studies that rely on plants collected from natural environments face serious challenges, including seasonal variation, genetic instability, pathogen influence, and lack of reproducibility. Biotechnology tools such as tissue culture provide solutions by ensuring the production of disease-free, genetically stable, and year-round plant material even with more quantity of secondary metabolites, while bioinformatics tools such as molecular docking, absorption, distribution, metabolism, excretion, and toxicity (ADMET) prediction, and network pharmacology allow rapid screening and validation of biactive compounds. Similarly, modern analytical techniques like high-performance liquid chromatography (HPLC), mass spectrometry (MS), and nuclear magnetic resonance (NMR) enable the precise isolation and characterization of bioactive compounds. Combining these approaches with in vitro and in vivo pharmacological testing establishes a reproducible, cost-effective, and time-saving avenue for antidiabetic drug discovery.

Future research should integrate in silico, in vitro, and in vivo studies using standardized plant materials from tissue culture. This approach will not only ensure reproducibility but also support biodiversity conservation. Ultimately, it provides a sustainable direction for continuous research aimed at developing more effective drugs for diabetes management.

REFERENCES

Akbari B, Baghaei-Yazdi N, Bahmaie M, Mahdavi Abhari F, (2021). The role of plant derived natural antioxidants in reduction of oxidative stress. BioFactors.

Akinmoladun, F. O., Komolafe, T. R., Olaleye, T. M., & Farombi, E. O. (2022). Pharmacological activities of *Bryophyllum pinnatum*: A review of ethnomedicinal, in vitro and in vivo studies. *Journal of Ethnopharmacology, 285*, 114897

Alam S, Sarker MMR, Sultana TN, Chowdhury MNR, Rashid MA, Chaity NI, Zhao C, Xiao J, Hafez EE, Khan SA and Mohamed IN (2022) Antidiabetic Phytochemicals from Medicinal Plants: Prospective Candidates for New Drug Discovery and Development. Front. Endocrinol. 13:800714. doi: 10.3389/fendo.2022.800714

Asmita Mahato, Goma Chaudhari, Gaurave Banjade, Shreesha Uprety (2023). Tissue Culture and Its Application in Modern Agriculture. i TECH MAG, Vol 4: 09-11

Bettoni, J.C.; Pathirana, R.; Bonnart, R.; Shepherd, A.; Volk, G. Cryopreservation of grapevine shoot tips from in vitro plants using droplet vitrification and V cryo-plate techniques. In Synthetic Seeds: Germplasm Regeneration, Preservation and Prospects; Faisal, M., Alatar, A.A., Eds.; Springer International Publishing: Cham, Switzerland, 2019; pp. 469–482

Chaudhary, K. K. & Mishra, N. A review on molecular docking: Novel tool for drug discovery. *JSM Chem.* **4**(3), 1029 (2016).

Chauhan D.S., Gupta P., Pottoo F.H., Amir M. Secondary metabolites in the treatment of diabetes mellitus: A paradigm shift. Curr. Drug Metab. 2020;21:493–511. doi: 10.2174/1389200221666200514081947.

Danova, K.; Marchioni, I.; Pistelli, L. Plant Tissue Culture and Secondary Metabolite Production Volume II. Plants 2023, 12, 3862. https://doi.org/10.3390/ plants12223862

Das DR, Kumar D, Kumar P, Dash BP. Molecular docking and its application in search of antisickling agent from Carica papaya. J Appl Biol Biotech 2020;8(01):105–116. DOI: 10.7324/JABB.2020.80117

Ezzat, S. M., El Bishbishy, M. H., Aborehab, N. M., Salama, M. M., Hasheesh, A., Motaal, A. A., & El-Khrisy, E. A. (2020). Upregulation of insulin receptor gene expression by quercetin and its impact on diabetes and cancer. *Journal of Food Biochemistry, 44*(7), e13217.

García, D. E., Narváez-Méndez, M., Morgan, S., Coronado-Malagon, M., Arce-Salinas, C. A., Barajas, A., Arenas, I., and Svarch, A. (2018). Biomarkers through the development, progression and chronic complications of diabetes mellitus: A mini-review. Journal of Endocrinology and Diabetes

Hameed, I., Masoodi, S. R., Mir, S. A., Nabi, M., Ghazanfar, K., & Ganai, B. A. (2015). Type 2 diabetes mellitus: From a metabolic disorder to an inflammatory condition. World Journal of Diabetes, 6(4), 598-612.

Kumari, P., Sharma, A. (2020). Plant tissue culture: Current status and opportunities. Biotechnology Reports, 27, e00536

Kundu, S., Salma, U., Ali, M. N., Hazra, A. K., and Mandal, N. (2018). Development of transgenic hairy roots and augmentation of secondary metabolites by precursor feeding in Sphagneticola calendulacea (L.) pruski. Ind. Crops Prod. 121, 206–215. doi: 10.1016/j.indcrop.2018.05.009

Lankatillake C, Huynh T, Dias DA. (2019). Understanding glycaemic control and current approaches for screening antidiabetic natural products from evidence-based medicinal plants. Plant Methods 15(1):1–35. doi: 10.1186/s13007-019-0487-8

Lenh V. V., Em Canh Pham, Cuong Viet Nguyen, Ngoc Thoi Nguyen Duong, Tuong Vi Le Thi, Tuyen Ngoc Truong. (2022). In vitro and in vivo antidiabetic activity, isolation of flavonoids, and in silico molecular docking of stem extract of Merremia tridentata (L.), Biomedicine & Pharmacotherapy, Volume 146, 2022, 112611, ISSN 0753-3322,

Lin X, Xu Y, Pan X, Xu J, Ding Y, Sun X, Song X, Ren Y, Shan PF (2020). Global, regional, and national burden and trend of diabetes in 195 countries and territories: an analysis from 1990 to 2025. Sci Rep. 2020 Sep 8;10(1):14790. doi: 10.1038/s41598-020-71908-9. PMID: 32901098; PMCID: PMC7478957.

Liu N, Wu XY, Song YD, Gao W, Huang LQ. Tissue culture of safflower and analysis of secondary metabolites in suspension cells]. Zhongguo Zhong Yao Za Zhi. 2021 Sep;46(17):4380-4388. Chinese. Doi:10.19540/j.cnki.cjcmm.20210523.105. PMID: 34581040.

Meng, X. Y., Zhang, H. X., Mezei, M. & Cui, M. (2011). Molecular docking: A powerful approach for structure-based drug discovery. *Curr. Comput. Aided Drug Des.* **7**, 146–157.

Mishra, A. (2015). Allelopathic properties of Lantana camara. Int. Res. J. Basic Clin. Study 3, 13–28. doi: 10.14303/irjbcs.2014.048

Mohiuddin AK, Chemi Mohiuddin AK. Chemistry of SecondaryMetabolites. Ann Clin Toxicol. 2019;2(1): 1014.

Morris, P., Carter, E. B., Hauck, B., Hughes, J. W., Allison, G., Theodorou, M. K. (2021). Responses of Lotus corniculatus to environmental change. 4: root carbohydrate levels at defoliation and regrowth climatic conditions are major drivers of phenolic content and forage quality. Planta 253 (2), 1–11. doi: 10.1007/s00425-020-03523-x

Ozyigit II, Dogan I, Hocaoglu-Ozyigit A, Yalcin B, Erdogan A, Yalcin IE, Cabi E and Kaya Y (2023) Production of secondary metabolites using tissue culture-based biotechnological applications. Front. Plant Sci. 14:1132555. doi:10.3389/fpls.2023.1132555

P. C. Agu, C. A. Afiukwa, O. U. Orji, E. M. Ezeh, I. H. Ofoke, C. O. Ogbu, E. I. Ugwuja & P. M. Aja. Molecular docking as a tool for the discovery of molecular targets of nutraceuticals in diseases management. Sci Rep 13, 13398 (2023). doi.org/10.1038/s41598-023-40160-2.

P. M. Aja, P. C. Agu, E. M. Ezeh, J. N. Awoke, H. A. Ogwoni, Tusubira Deusdedit, E. U. Ekpono, I. O. Igwenyi, E. U. Alum, E. I. Ugwuja, A. U. Ibiam, C. A. Afiukwa & Abayomi Emmanuel Adegboyega (2021). Prospecting into therapeutic potentials of *Moringa oleifera* phytocompounds against cancer upsurge: De novo synthesis of test compounds, molecular docking, and ADMET studies. *Bull. Natl. Res. Cent.* 45, 99.

Patel, D. K., & Mishra, S. (2021). Antidiabetic potential of medicinal plants and their bioactive compounds: A review. Biomedicine & Pharmacotherapy, 139, 111595

Piatczak, E., Jeleń, A., Makowczyńska, J., Zielińska, S., Kuźma, Ł., and Balcerczak, E. (2019). Establishment of hairy root cultures of Rehmannia elata NE brown ex prain and production of iridoid and phenylethanoid glycosides. Ind. Crops Prod. 137, 308–314. doi: 10.1016/j.indcrop.2019.05.022

Pinzi, L. & Rastelli, G. Molecular docking: Shifting paradigms in drug discovery. *Int. J. Mol. Sci.* **20**, 4331 (2019).

Potluri, H., Prasanth, D. S. & Atmakuri, L. R. (2021). In vivo antinociceptive effect of methanolic extract of *Ipomoea marginata* Desr. in rodents as well as in silico molecular docking of some phytoconstituents from the plant. *Indian J. Pharm. Sci.* **83**(4), 732–741

Riahi, L., Chograni, H., Ben Rejeb, F., Ben Romdhane, M., Masmoudi, A. S., and Cherif, A. (2022). Efficient in vitro regeneration of the endangered species Artemisia arborescens l. through direct organogenesis and impact on secondary metabolites production. Horticult. Environment Biotechnol. 63 (3), 439–450. doi: 10.1007/s13580- 021-00400-8

Saheed Olatunbosun Akiode 1, Adewale Elijah Fadeyi 2, Anna Asheahe Ibi and Muhammad Bashir Hassan (2021). Effect of seasonal changes on the quantity of secondary metabolites from neem and eucalyptus plants in North Central Nigeria. World Journal of Biology Pharmacy and Health Sciences, 2021, 07(02), 043–052

Sahoo, R. N., Pattanaik, S., Pattnaik, G., Mallick, S. & Mohapatra, R. (2022). Review on the use of molecular docking as the first line tool in drug discovery and development. *Indian J. Pharm. Sci.* 84(5), 1334–1337

Sangwan, N. S., Mishra, L. N., & Sangwan, R. S. (2021). Withanolides from *Withania somnifera* and their role in diabetes management: An overview. *Phytomedicine, 85*, 153546.

Sehgal, D., Khan, T. (2020). "Plant tissue culture: beyond being a tool for genetic engineering," in Environmental microbiology and biotechnology. Eds. Singh, A., Srivastava, S., Rathore, D., Pant, D. (Singapore: Springer), 175–200

Setyani W, Murwanti R, Sulaiman TN,Hertiani T. Flavonoid from Moringa oleifera leaves revisited:A review article on in vitro, in vivo, and in silico studies ofantidiabetic insulin-resistant activity. J Adv Pharm Technol Res2023;14:283-8.

Shahzad A, Liu W, Hussain S , Yueli N., Kun C., Yijian S., Xiangjie L., Qiuxin D., Jiaojiao X., Jinshan Z., Zhe X., Buqing S., Yuechun Z., Qiao Z., and ZheYang (2025) Integrated in vitro, in silico, andin vivo approaches to elucidate the antidiabetic mechanisms of Cicerarietinum and Hordeum vulgare extract and secondary metabolites. SciRep 15:6620. doi.org/10.1038/s41598-025-89642-524. Kwok JJN,

Singh, K. D., Chetia, D., Gogoi, N., Gogoi, B., & Rudrapal, M. (2024). In vivo and in silico based evaluation of antidiabetic potential of an isolated flavonoid from *Allium hookeri* in type 2 diabetic rat model. *Chemistry & Biodiversity, 21*(1), e202301299.

Singh, T., Yadav, R., and Agrawal, V. (2020). Effective protocol for isolation and marked enhancement of psoralen, daidzein and genistein in the cotyledon callus cultures of Cullen corylifolium (L.) medik. Ind. Crops Prod. 143, 111905. doi: 10.1016/ j.indcrop.2019.111905

Sun C., Zhao C., Guven E.C., Paoli P., Simal-Gandara J., Ramkumar K.M., Wang S., Buleu F., Pah A., Turi V. Dietary polyphenols as antidiabetic agents: Advances and opportunities. Food Front. 2020;1:18–44. doi: 10.1002/fft2.15.

Wojciechowska, M., Owczarek, A., Kiss, A. K., Grabkowska, R., Olszewska, M. A., □ and Grzegorczyk-Karolak, I. (2020). Establishment of hairy root cultures of Salvia Ozyigit *et al*. 10.3389/fpls.2023.1132555 Frontiers in Plant Science 27 frontiersin.org bulleyana diels for production of polyphenolic compounds. J. Biotechnol. 318, 10–19. doi: 10.1016/j.jbiotec.2020.05.002

Wu, F., Zhou, Y,. Li, L,. Anhuan, X., Shen., Chen G., Wang, X., Liang, X., Tan, M., Huang 1, Z., (2020). Computational approaches in preclinical studies on drug discovery: ADMET prediction methods. *Frontiers in Pharmacology*. (PMC)

Yousefian, S., Lohrasebi, T., Farhadpour, M., and Haghbeen, K. (2020). Production of phenolic acids in hairy root cultures of medicinal plant Mentha spicata l. @ in response to elicitors. Mol. Biol. Res. Commun. 9 (1), 23. doi: 10.22099% 2Fmbrc.2020.36031.1475