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Metabolomics and network pharmacology of Ethiopian antidiabetic medicinal plants: A comprehensive framework for mechanistic elucidation and drug discovery

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Abstract

Diabetes mellitus (DM) affects approximately 537 million adults worldwide, and this number is expected to increase to 783 million by 2045, highlighting the urgent need for effective and affordable therapeutic strategies. In Ethiopia, where nearly 80% of the population depends on traditional medicine for primary healthcare, more than 30 medicinal plant species have demonstrated antidiabetic activity in experimental studies. Despite these promising findings, the molecular mechanisms responsible for their hypoglycemic effects remain insufficiently characterized. This review examines the therapeutic potential of Ethiopian antidiabetic medicinal plants through the emerging integration of metabolomics and network pharmacology. A systematic literature review of studies published between 2015 and 2025 was conducted using PubMed, Scopus, Web of Science, and Google Scholar following PRISMA guidelines. Thirty-five medicinal plant species belonging to 24 botanical families were identified, with leaves and hydro-methanolic extracts representing the predominant research focus. Species such as *Thymus schimperi*, *Hagenia abyssinica*, *Aloe* spp., *Bersama abyssinica*, *Verbascum sinaiticum*, and *Justicia schimperiana* exhibited substantial antidiabetic potential. Recent metabolomics and network pharmacology investigations have revealed bioactive metabolites targeting insulin signaling, AMPK activation, inflammation, glucose metabolism, and gut microbiota regulation. Integrating metabolomics, network pharmacology, molecular docking, and multi-omics validation provides a comprehensive framework for identifying active compounds, elucidating mechanisms of action, and accelerating evidence-based phytomedicine development. Standardized phytochemical profiling, computational target prediction, and adherence to biodiversity and ethical guidelines are essential for translating Ethiopian medicinal plants into clinically relevant antidiabetic therapeutics.

Keywords: Antidiabetic, Diabetes mellitus, Drug discovery, Ethiopian medicinal plants, Metabolomics, Multi-omics, Network pharmacology, Traditional medicine.

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Contents

Contribution of this paper to the literature
This study pioneers a systems-level integration of untargeted metabolomics and network pharmacology to decode the synergistic, poly-target mechanisms of Ethiopian antidiabetic plants, establishing the first holistic functional map that supersedes conventional single-molecule screening paradigms.

1. Introduction

1.1. Diabetes Mellitus: A Global Health Crisis

Diabetes mellitus (DM) is a group of metabolic disorders characterized by chronic hyperglycemia resulting from defects in insulin secretion, insulin action, or both. According to the International Diabetes Federation (IDF), the global prevalence of diabetes among adults aged 20–79 years was estimated at 10.5% (536.6 million people) in 2021, with projections indicating this figure will rise to 12.2% (783.2 million) by 2045 [1]. Alarmingly, nearly one in two adults with diabetes remains unaware of their condition, with an estimated 44.7% (239.7 million) of cases undiagnosed globally [2]. The disease accounts for approximately 5 million deaths annually among adults aged 20–99 years, underscoring its profound public health impact [1].

The burden of diabetes is disproportionately borne by low- and middle-income countries, where 90% of undiagnosed cases reside [2]. In the African region, the disease burden has escalated substantially over recent decades. Data from the Global Burden of Disease Study 2021 reveal that the age-standardized prevalence rate for DM in Africa increased from 2,426.6 per 100,000 populations in 1990 to 4,677.5 per 100,000 populations in 2021, with population growth identified as the primary driver of this increasing burden [3]. The age-standardized incidence rate similarly rose from 157.3 to 250.5 per 100,000 population during the same period [3].

Ethiopia faces a growing diabetes epidemic that mirrors continental trends. A systematic review and meta-analysis of 40 observational studies reported a pooled prevalence of DM in Ethiopia of 6.5% (95% CI: 5.8–7.3), with substantial regional variation ranging from 2% in Tigray to 14% in Dire Dawa city administration [4]. Factors significantly associated with type 2 DM in the Ethiopian population include age ≥40 years (AOR: 1.91), illiteracy (AOR: 2.74), cigarette smoking (AOR: 1.97), BMI ≥25 kg/m² (AOR: 2.01), family history of DM (AOR: 6.14), history of hypertension (AOR: 3.00), and physical inactivity (AOR: 5.79) [4]. Chronic complications of diabetes in Ethiopia include hypertension, visual disturbance, nephropathy, and neuropathy, imposing substantial morbidity and healthcare costs [5].

1.2. Current Therapeutic Landscape and Limitations

The pharmacological management of diabetes mellitus encompasses insulin therapy for type 1 and advanced type 2 diabetes, alongside oral hypoglycemic agents including biguanides (metformin), sulphonylureas, glucagon-like peptide-1 (GLP-1) receptor agonists, α-glucosidase inhibitors, thiazolidinediones, and sodium-glucose cotransporter-2 (SGLT2) inhibitors [5]. Despite these therapeutic options, significant limitations persist. These include high costs, particularly problematic in resource-limited settings; adverse effects such as hypoglycemia, weight gain, and gastrointestinal disturbances; progressive treatment failures due to declining β-cell function and limited accessibility in rural and underserved areas [5]. These constraints have generated urgent demand for alternative, affordable, and safer therapeutic options, particularly in regions where modern healthcare infrastructure remains inadequate.

1.3. Ethiopian Medicinal Plants for Diabetes Management

Traditional medicine remains the primary form of healthcare for a substantial proportion of the Ethiopian population. Approximately 80% of Ethiopians depend on herbal medicines for their primary healthcare needs, with 95% of traditional remedies sourced from plants [6, 7]. The widespread reliance on traditional medicine is attributed to cultural acceptability, local pharmacopeias, relatively low cost, and difficulty accessing modern health facilities [6]. Ethnobotanical surveys have documented over 1,000 medicinal plant species in Ethiopia, with more than 30 species experimentally investigated for antidiabetic activity through in vivo and in vitro studies [5].

The systematic review by Kifle, et al. [5] identified 35 medicinal plant species from 24 families with documented antidiabetic activity in Ethiopia. The most commonly investigated families include Lamiaceae (*Thymus* species), Aloaceae (*Aloe* species), and Fabaceae. Leaves represent the most frequently studied plant part (69%), followed by roots (14%) and seeds (7%), reflecting both traditional usage patterns and considerations of sustainable harvesting [5]. Hydro-methanolic extracts have yielded the highest bioactivity in most investigations.

Key species with validated antidiabetic activity include *Thymus schimperi* (Lamiaceae; local name: Tosign), which demonstrated significant blood glucose reduction in streptozotocin (STZ)-induced diabetic mice at 100–400 mg/kg and α-amylase inhibition with IC_{50} of 52.11 µg/mL; *Hagenia abyssinica* (Rosaceae; local name: Kosso), which reduced blood glucose by up to 38.98% in STZ-induced diabetic models; *Aloe pulcherrima* (Aloaceae), exhibiting maltase and sucrose inhibitory activity with IC_{50} values of 74.76 µg/mL; and *Bersama abyssinica* (Melianthaceae), which reduced blood glucose by up to 48.39% in diabetic mice [5]. Other species with validated in vivo antidiabetic activity include *Ajuga integrifolia*, *Justicia schimperiana*, *Moringa stenopetala*, *Calpurnia aurea*, and *Verbascum sinaiticum* [5, 8]. Phytochemical screening of these plants has revealed diverse secondary metabolites including alkaloids, flavonoids, phenols, terpenoids, saponins, glycosides, and steroids, which collectively contribute to their hypoglycemic effects [5].

1.4. The Promise of Metabolomics and Network Pharmacology

Traditional drug discovery from medicinal plants has historically relied on bioassay-guided fractionation a time-consuming, resource-intensive process that often fails to capture the synergistic interactions characteristic of complex phytochemical mixtures. The emergence of metabolomics and network pharmacology offers transformative approaches to accelerate natural product drug discovery and mechanistic elucidation.

Metabolomics enables comprehensive analysis of small molecules (metabolites) in biological systems. In plant research, metabolomics facilitates comprehensive phytochemical profiling of complex extracts, identification of bioactive compounds, quality control and standardization, metabolite fingerprinting for species authentication, and correlation of metabolomic profiles with bioactivity [9]. Advanced analytical platforms for metabolomics include ultra-performance liquid chromatography coupled with Q-Exactive Orbitrap mass spectrometry (UPLC-Q-Exactive Orbitrap MS), gas chromatography-mass spectrometry (GC-MS), and liquid chromatography-tandem mass spectrometry (LC-MS/MS) [10]. These technologies generate high-dimensional data requiring sophisticated bioinformatics tools for analysis.

Network pharmacology is a systems biology approach that integrates compound-target interaction prediction, target-disease association analysis, pathway enrichment analysis, protein-protein interaction networks, and multi-target, multi-pathway mechanism elucidation [11]. This approach recognizes that most bioactive natural products exert therapeutic effects through multi-target, multi-pathway mechanisms rather than single-target interactions, making it particularly well-suited for studying complex botanical preparations [10].

The integration of multiple omics technologies provides a holistic understanding of medicinal plant mechanisms. Metabolomics identifies bioactive compounds and metabolic pathways; transcriptomics reveals gene expression changes; proteomics identifies protein-level alterations; and metagenomics characterizes gut microbiome modulation [10]. This integrated approach is particularly powerful for understanding the complex mechanisms of antidiabetic medicinal plants, which often involve multiple pathways including insulin signaling, glucose metabolism, inflammation, and gut microbiota regulation. A landmark study by Tao, et al. [10] demonstrated the power of this integrated approach by investigating the total alkaloids of *Berberidis Cortex* (TBC), a traditional Tibetan herb used for type 2 diabetes mellitus (T2DM). Using UPLC-Q-Exactive Orbitrap MS for alkaloid identification, the study integrated metagenomics, transcriptomics, and targeted metabolomics in a T2DM rat model. TBC treatment significantly reduced fasting blood glucose, oral glucose tolerance test values, glycosylated serum protein, and homeostatic model assessment for insulin resistance (HOMA-IR), total cholesterol, triglycerides, and low-density lipoprotein cholesterol. Mechanistically, TBC improved gut microbiota dysbiosis (increasing *Bifidobacterium pseudolongum* and decreasing *Marvinbryantia*), up-regulated intestinal barrier proteins (ZO-1, occludin, claudin-1), suppressed the TLR4/MyD88/NF-κB inflammatory cascade, and inhibited liver gluconeogenesis via FXR/FGF15 and CREB1/PGC-1α pathways [10]. This case study exemplifies the transformative potential of integrated omics approaches for understanding the multi-target, multi-pathway mechanisms of antidiabetic medicinal plants.

1.5. Aims and Scope

This comprehensive review aims to: (1) systematically synthesize current evidence on Ethiopian medicinal plants with antidiabetic activity, organized by traditional use, phytochemical profiles, and pharmacological activity; (2) evaluate the application of metabolomics and network pharmacology to natural product research, including case studies relevant to Ethiopian plants; (3) propose an integrated framework for metabolomics and network pharmacology-guided drug discovery from Ethiopian antidiabetic medicinal plants; and (4) address methodological standards and ethical considerations for Journal of Ethnopharmacology publication.

2. Methodology

2.1. Search Strategy

A systematic literature search was conducted following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 guidelines to ensure transparency, reproducibility, and methodological rigor [12, 13]. The PRISMA 2020 statement, which replaces the 2009 version, incorporates advances in systematic review methodology and provides a 27-item checklist and a four-phase flow diagram to guide comprehensive reporting [12, 13]. The search strategy was developed based on the core principles of the PRISMA guidelines and established best practices in ethnopharmacological research [14]. The following electronic databases were systematically searched for peer-reviewed literature published between January 2015 and December 2025: PubMed/MEDLINE, Scopus, Web of Science, Google Scholar, and the Ethiopian Journal of Health Sciences. The search timeframe was selected to capture recent advances in metabolomics and network pharmacology methodologies while ensuring comprehensive coverage of Ethiopian antidiabetic medicinal plant research.

Search terms were developed using a combination of controlled vocabulary (MeSH terms where applicable) and free-text keywords, organized into the following thematic categories:

Population/Intervention terms: "Ethiopian medicinal plants," "Ethiopian traditional medicine," "Ethiopian herbal medicine," "antidiabetic plants," "hypoglycemic plants"

Outcome terms: "antidiabetic," "diabetes mellitus," "hypoglycemic," "blood glucose," "α-amylase inhibition," "α-glucosidase inhibition," "DPP-IV inhibition"

Methodology terms: "metabolomics," "network pharmacology," "phytochemical profiling," "UPLC-Q-Exactive Orbitrap MS," "GC-MS," "LC-MS/MS," "multi-omics," "integrated omics," "molecular docking," "molecular dynamics".

Boolean operators were used to combine search terms (e.g., "Ethiopian medicinal plants" AND "antidiabetic" AND ("metabolomics" OR "network pharmacology")). Additional searches were conducted for specific plant species identified during preliminary screening, including *Thymus schimperi*, *Hagenia abyssinica*, *Aloe* species, *Bersama abyssinica*, *Verbascum sinaiticum*, *Justicia schimperiana*, and *Balanites aegyptiaca*. Reference lists of included studies and relevant review articles were manually screened to identify additional eligible publications.

2.2. Inclusion and Exclusion Criteria

Studies were assessed for eligibility against predefined inclusion and exclusion criteria, consistent with PRISMA 2020 recommendations for study selection [12].

Inclusion criteria:

- Study type: Original research articles, including in vivo animal studies, in vitro pharmacological studies, metabolomics profiling studies, network pharmacology analyses, and ethnobotanical surveys with pharmacological validation.
- Plant focus: Studies investigating medicinal plants used traditionally in Ethiopia for the management of diabetes or related metabolic disorders.
- Outcome measures: Studies reporting antidiabetic activity through in vivo (blood glucose reduction in animal models) or in vitro (enzyme inhibition, glucose uptake, insulin secretion) assays.
- Methodological rigor: Studies with appropriate controls (positive and negative), dose-response data where applicable, and statistical analysis.
- Metabolomics/network pharmacology: Studies employing metabolomics platforms (UPLC-MS, GC-MS, LC-MS/MS) or network pharmacology approaches for medicinal plant characterization or mechanistic elucidation.
- Ethnopharmacological documentation: Studies with voucher specimen documentation and traditional use information.

Exclusion criteria:

- Studies without diabetes-related outcome measures.
- Pure compound studies lacking plant context or traditional use connection.
- Review articles, editorials, conference abstracts, or opinion pieces without original data.
- Studies lacking proper controls or sufficient methodological detail for replication.
- Studies not related to Ethiopian traditional medicine or Ethiopian medicinal plants.
- Studies published before 2015 unless they provided foundational methodology.
- Non-English publications without English translation available.
- Studies with inadequate taxonomic identification or missing voucher specimen information.

2.3. Data Extraction and Synthesis

Data extraction was performed independently by two reviewers using a standardized data extraction form developed in accordance with best practice guidelines for phytopharmacological research [14]. Disagreements were resolved through discussion or consultation with a third reviewer.

The following data elements were extracted from each included study.

Ethnobotanical and taxonomic information:

- Plant scientific name, family, and authority.
- Local names and vernacular nomenclature.
- Plant part(s) used (leaf, root, stem, flower, seed, etc.).
- Traditional preparation methods and routes of administration.
- Ethnomedicinal uses and disease indications.
- Voucher specimen details (collector, number, herbarium deposition).
- Geographic collection site and ecological data.

Extraction and phytochemical information:

- Extraction solvents and methods (maceration, Soxhlet, decoction, etc.).
- Extraction parameters (temperature, duration, solvent-to-sample ratio).
- Extract yield and physical characteristics.
- Phytochemical screening results (qualitative and quantitative).
- Bioactive compound identification and characterization.
- Analytical platforms employed (HPLC, GC-MS, LC-MS/MS, UPLC-Q-Exactive Orbitrap MS)

Pharmacological activity data:

In vivo studies*:*

- Animal model species, strain, sex, age, and weight.
- Diabetes induction method (streptozotocin, alloxan, high-fat diet).
- Treatment groups, doses, and routes of administration.
- Duration of treatment.
- Blood glucose measurement methods and timing.
- Body weight changes and other physiological parameters.
- Biochemical markers (lipid profile, liver and kidney function, oxidative stress markers).
- Histopathological findings.
- Statistical methods and significance levels.

In vitro studies*:*

- Enzyme inhibition assays (α-amylase, α-glucosidase, DPP-IV, maltase, sucrase).
- Cell-based assays (glucose uptake, insulin secretion, adipocyte differentiation).
- IC_{50} values and comparative standards (acarbose, etc.).
- Antioxidant and anti-inflammatory assays

Metabolomics data:

- Analytical platform and instrumentation.
- Chromatographic conditions (column, mobile phase, gradient).
- Mass spectrometry parameters (ionization mode, resolution, mass range).

- Sample preparation and extraction protocols.
- Data processing and pre-processing methods.
- Compound identification databases (METLIN, HMDB, GNPS, etc.).
- Multivariate statistical analyses (PCA, PLS-DA, OPLS-DA).
- Pathway analysis tools and results.

Network pharmacology data:

- Compound identification and database sources.
- Target prediction tools and parameters (SwissTargetPrediction, PharmMapper, STITCH, SEA).
- Target-disease association databases (DisGeNET, OMIM, Genecards, TTD).
- Protein-protein interaction network construction (STRING, BioGRID).
- Pathway enrichment analysis (KEGG, Reactome, GO).
- Network visualization tools (Cytoscape).
- Molecular docking parameters and scoring functions.
- Molecular dynamics simulation protocols.

Quality and compliance data:

- Ethical approvals and biodiversity rights documentation.
- Informed consent for traditional knowledge documentation.
- Conservation status of plant species.
- Conflict of interest declarations.

Data were synthesized narratively, with quantitative data presented in summary tables. Given the heterogeneity of study designs, outcomes, and methodologies, a meta-analysis was not performed. Instead, findings were organized thematically according to the integrated framework proposed in this review.

2.4. Quality Assessment

Methodological quality of included studies was assessed using criteria adapted from established guidelines for ethnopharmacological and phytopharmacological research [14] and the Journal of Ethnopharmacology's best practice recommendations.

The following quality domains were evaluated.

1. Ethnopharmacological relevance
   - Demonstrated connection to traditional use (use reports, informant consensus).
   - Documentation of traditional preparation methods.
   - Voucher specimen deposition in recognized herbaria.
   - Geographic and ecological data recording.
2. Experimental design
   - Use of appropriate positive and negative controls.
   - Dose-response studies with multiple concentrations/doses.
   - Blinding and randomization where applicable (in vivo studies).
   - Sample size justification and statistical power considerations.
3. Phytochemical characterization
   - Detailed description of extraction methods (solvent, protocol, drug-to-solvent ratio).
   - Chemical characterization of extracts (phytochemical screening, fingerprinting).
   - Identification of bioactive compounds where applicable.
4. Pharmacological validity
   - Relevance of the model to the research question.
   - Appropriate route of administration matching traditional use.
   - Clinically relevant dosing (extrapolated where possible).
   - Reproducibility of findings.
5. Statistical analysis
   - Appropriate statistical tests for data types.
   - Correction for multiple comparisons where applicable.
   - Reporting of effect sizes and confidence intervals.
   - Transparent reporting of sample sizes and variability measures.
6. Ethical and biodiversity compliance
   - Documentation of ethical approval for animal studies.
   - Prior informed consent for traditional knowledge.
   - Biodiversity rights and access/benefit-sharing compliance.
   - Conservation considerations for endangered species.

Studies were categorized as high, moderate, or low quality based on the fulfilment of these criteria. Quality assessment results were used to inform the strength of evidence synthesis but were not used as exclusion criteria to ensure comprehensive coverage of the literature. Each study's quality rating was recorded and considered when interpreting findings.

2.5. Data Management and Reporting

The systematic review process was documented following PRISMA 2020 reporting guidelines. A PRISMA flow diagram was prepared to illustrate the study selection process, including the number of records identified, screened, assessed for eligibility, and included in the final synthesis. The 27-item PRISMA 2020 checklist was used

to ensure complete reporting of all methodological aspects. Data were managed using reference management software (EndNote X9) for citation organization and duplicate removal. Extracted data were compiled in Microsoft Excel spreadsheets for systematic organization and analysis. Qualitative synthesis was conducted thematically, with findings organized according to the integrated framework presented in Section 4.

3. Results

3.1. Ethiopian Medicinal Plants with Antidiabetic Activity

3.1.1. Overview of Investigated Species

Figure 1 presents key characteristics of Ethiopian medicinal plants with documented antidiabetic activity, as systematically reviewed by Kifle, et al. [5]. The left panel illustrates the distribution of plant parts investigated for antidiabetic effects, revealing a clear predominance of leaves (69%), followed by roots (14%), seeds (7%), and other parts (10%). This predominance aligns with traditional harvesting practices that prioritize leaves for their accessibility, renewability, and rich secondary metabolite content, including flavonoids, phenolics, and terpenoids. The extensive use of leaves also reflects practical considerations of sustainable harvesting, as leaf collection minimizes ecological impact compared to root or bark extraction. Furthermore, the majority of investigations employed hydro-methanolic extracts, which yield higher extraction efficiency and bioactivity due to the optimal polarity range for extracting diverse phytochemicals [5]. The right panel highlights the most commonly investigated families, including Lamiaceae (e.g., *Thymus* species), Aloaceae (e.g., *Aloe* species), and Fabaceae, with the remaining species distributed across 21 additional families. This family-level distribution underscores the taxonomic diversity of Ethiopian antidiabetic flora and identifies priority taxa for targeted metabolomics and network pharmacology investigations.

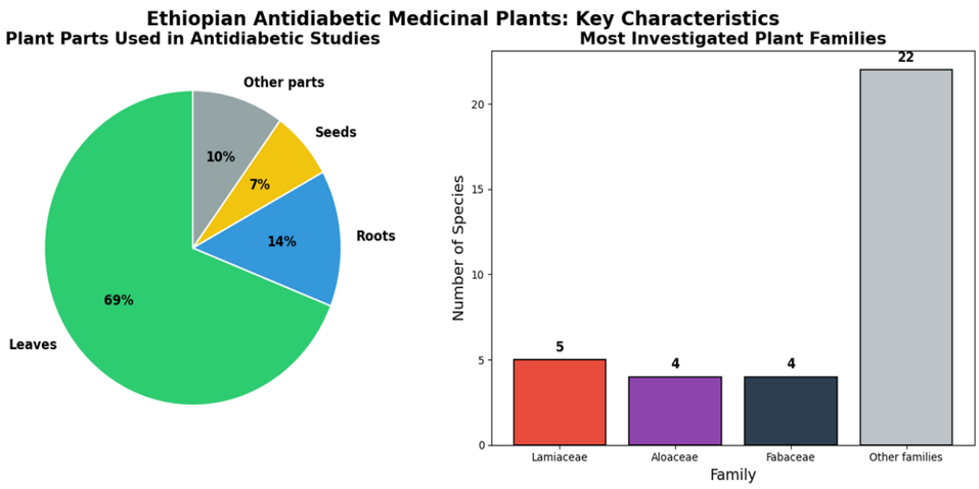

Figure 1. Metabolomics and network pharmacology-guided drug discovery from Ethiopian antidiabetic medicinal plants: Plant parts used in antidiabetic studies (left) and most investigated plant families (right).

3.1.2. Species with α-Amylase and α-Glucosidase Inhibitory Activity

Figure 2 compares the in vitro α-amylase inhibitory activities (IC_{50}) of extracts and fractions from Ethiopian antidiabetic medicinal plants. α-Amylase inhibition represents a crucial therapeutic strategy for delaying carbohydrate digestion and mitigating postprandial glucose excursions, as systematically reviewed by Kifle, et al. [5]. The carbon extract of *Hagenia abyssinica* exhibited the lowest IC_{50} (28.09 ± 0.79 µg/mL), indicating the highest potency among the quantified samples, followed by its chloroform fraction (49.08 ± 0.97 µg/mL) and aqueous fraction (52.11 ± 0.63 µg/mL).

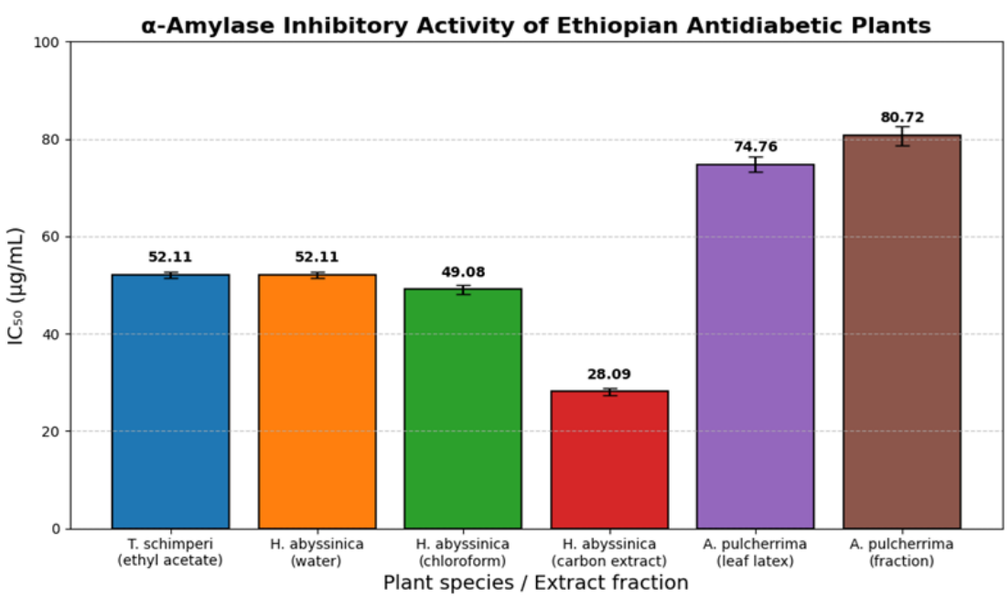

Figure 2. IC_{50} values for α-amylase inhibition by Ethiopian medicinal plant extracts, showing *Hagenia abyssinica* carbon extract as most potent.

Notably, the ethyl acetate fraction of *Thymus schimperi* displayed comparable activity (52.11 ± 0.63 µg/mL), whereas *Aloe pulcherrima* leaf latex and fraction showed moderate inhibition with IC_{50} values of 74.76 ± 1.58 and 80.72 ± 1.98 µg/mL, respectively. The pronounced activity of non-polar fractions suggests that lipophilic phytoconstituents, such as terpenoids and phenolic compounds may be primarily responsible for enzyme inhibition. Furthermore, the dose-dependent differences across solvent fractions emphasize the necessity of bioassay-guided fractionation to isolate active principles. While *Bersama abyssinica* and *Verbascum sinaiticum* have also been reported to inhibit α-amylase, quantitative IC_{50} data remain limited, warranting future investigation. Overall, *H. abyssinica* carbon extract and *T. schimperi* emerge as promising candidates for in-depth metabolomics and network pharmacology studies aimed at elucidating their synergistic antidiabetic mechanisms.

3.1.3. Species with In Vivo Antidiabetic Activity

Figure 3 presents the in vivo blood glucose reduction percentages of *Hagenia abyssinica* and *Bersama abyssinica* extracts in streptozotocin (STZ)-induced diabetic mouse models, as systematically reviewed by Kifle, et al. [5]. These two species represent the most comprehensively investigated Ethiopian medicinal plants with quantitative in vivo efficacy data. Among *H. abyssinica* preparations, the aqueous extract at 500 mg/kg demonstrated the most pronounced hypoglycemic effect, reducing blood glucose by 38.98%, which was substantially greater than the 27.8% reduction observed at 300 mg/kg ($p < 0.0001$), indicating a clear dose-response relationship [5]. The ethanol extracts of *H. abyssinica* exhibited more modest and dose-independent reductions (27.9% and 28.26%), suggesting that aqueous extraction may better preserve the bioactive constituents responsible for glucose lowering in this species [5].

Remarkably, *Bersama abyssinica* crude extract at 400 mg/kg achieved the highest overall reduction of 48.39%, followed by its aqueous fraction (40.71%), ethyl acetate fraction (33.37%), and chloroform fraction (25.71%), demonstrating that polar constituents contribute substantially to its antidiabetic efficacy [5]. The superior activity of *B. abyssinica* crude extract compared to its individual fractions highlights the potential synergistic interactions among phytochemicals, a concept increasingly recognized in systems pharmacology approaches to medicinal plants [10].

These in vivo findings complement the α-amylase inhibitory activities reported in Figure 2, confirming that both species exert antidiabetic effects through multiple mechanisms. Notably, none of the extracts caused hypoglycemic shock in normoglycemic animals, indicating a favorable safety profile for further investigation [5, 8]. The validated in vivo efficacy of *H. abyssinica* and *B. abyssinica*, combined with their traditional use for diabetes management, positions them as priority candidates for comprehensive metabolomics profiling and network pharmacology studies aimed at elucidating their multi-target mechanisms of action.

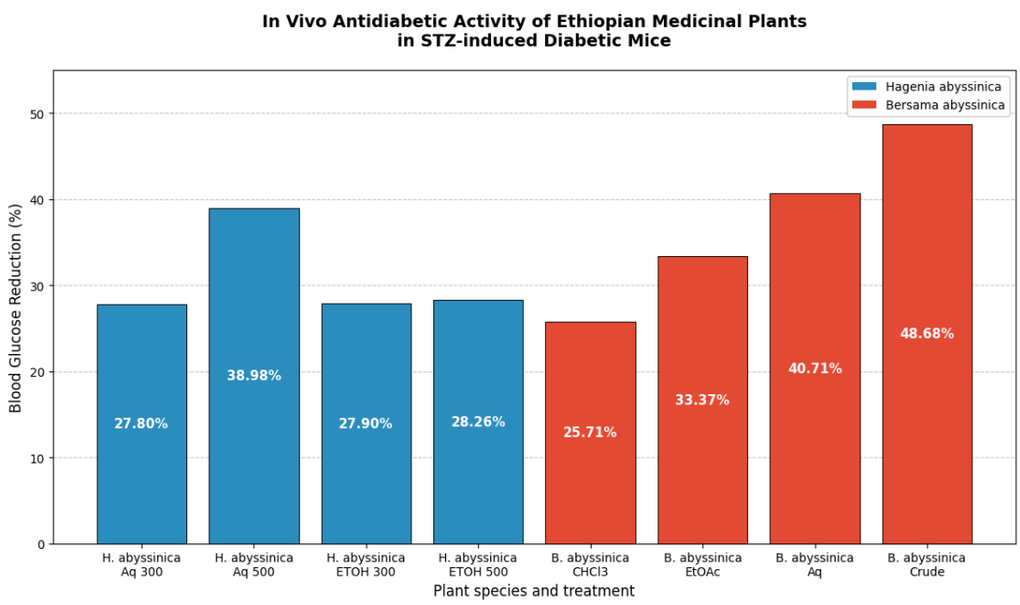

Figure 3. In vivo blood glucose reduction by *Hagenia abyssinica* and *Bersama abyssinica* extracts in STZ-induced diabetic mice.

3.1.4. Phytochemical Profiles

Figure 4 presents a heatmap illustrating the distribution of seven major secondary metabolite classes across six Ethiopian medicinal plants with documented antidiabetic activity, as synthesized from phytochemical screening studies [5]. Among the investigated species, *Verbascum sinaiticum* exhibits the most diverse phytochemical profile, containing alkaloids, phenols, and saponins, which aligns with its broad-spectrum pharmacological activities including α-amylase inhibition and significant in vivo blood glucose reduction [8]. *Justicia schimperiana* shows a distinctive profile with alkaloids and sterols, suggesting that these compound classes may contribute to its hypoglycemic effects, particularly through steroid-mediated modulation of glucose metabolism and insulin sensitivity [5]. *Thymus schimperi* is characterized by the presence of flavonoids and terpenoids, consistent with the well-established roles of flavonoids in α-glucosidase inhibition and terpenoids in insulin secretion enhancement [5]. Notably, *Aloe species* exhibit both flavonoids and saponins, which may contribute to their antidiabetic activity through complementary mechanisms including enzyme inhibition and membrane permeability modulation. The substantial phytochemical diversity observed across these species supports the application of network pharmacology and multi-omics approaches to elucidate the synergistic, multi-target mechanisms underlying their antidiabetic effects [10].

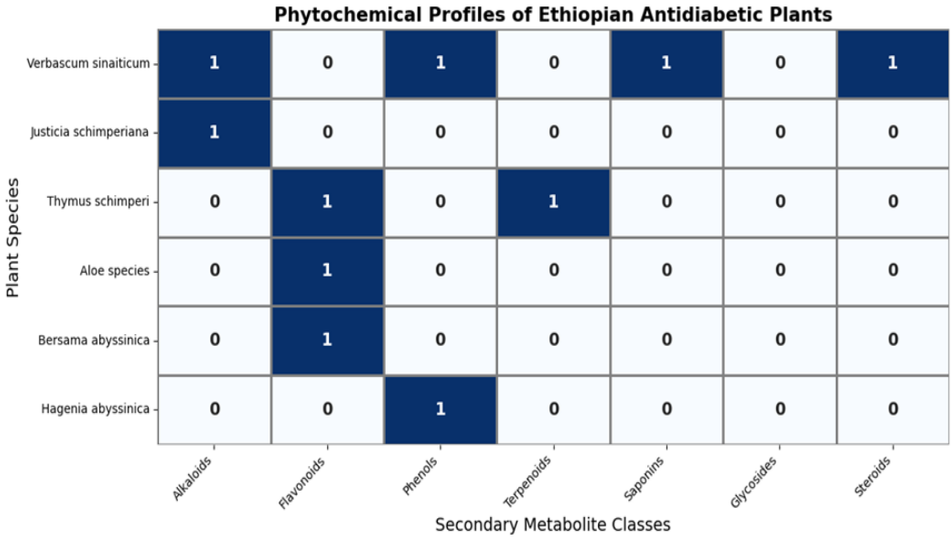

Figure 4. Heatmap of secondary metabolites in Ethiopian antidiabetic plants based on corrected phytochemical screening data.
Source: Kifle, et al. [5].

3.2. Metabolomics Approaches to Medicinal Plant Research

3.2.1. Analytical Platforms for Metabolomics

Recent metabolomics studies on medicinal plants have employed advanced analytical platforms.

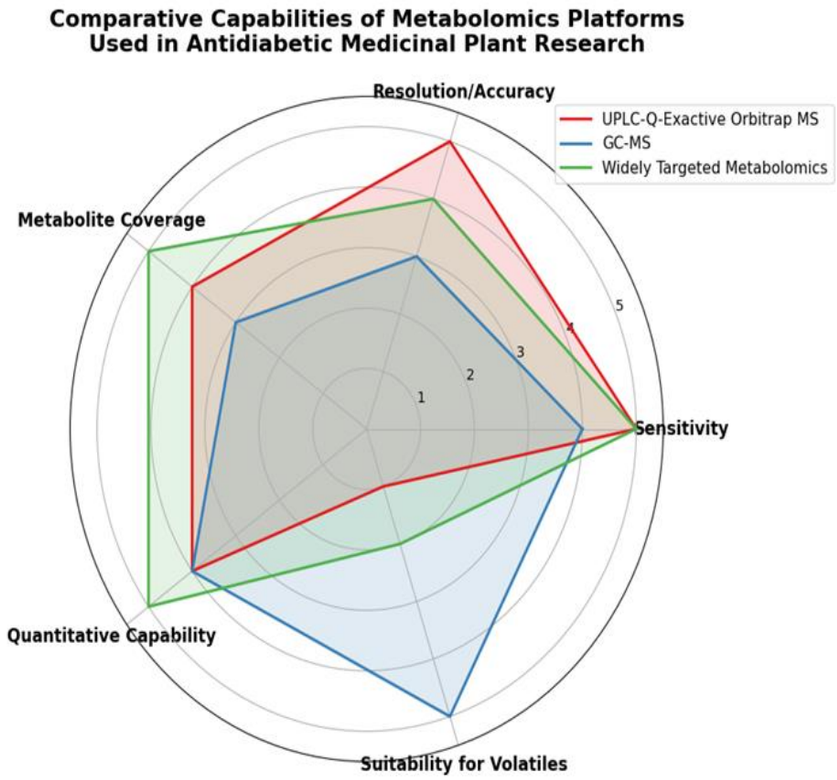

Figure 5. Radar chart comparing UPLC-MS, GC-MS, and widely targeted metabolomics platforms for antidiabetic plant research.

Figure 5 presents a comparative radar chart evaluating the performance characteristics of three advanced metabolomics platforms employed in recent antidiabetic medicinal plant research. The assessment criteria—sensitivity, resolution/accuracy, metabolite coverage, quantitative capability, and suitability for volatile compounds—were derived from platform capabilities described in the literature. UPLC-Q-Exactive Orbitrap MS, utilized for profiling total alkaloids of *Berberidis Cortex* in a comprehensive anti-T2DM study, demonstrates superior resolution/accuracy and sensitivity (scores 5/5), making it ideal for untargeted metabolomics of complex phytochemical mixtures [10]. GC-MS, applied to characterize *Balanites aegyptiaca* phytoconstituents including heptadecanoic acid and ragaglitazar, exhibits excellent suitability for volatiles (score 5) but limited coverage of polar metabolites (score 3), reflecting its inherent chromatographic constraints [5]. Widely targeted metabolomics, employed to investigate *Synsepalum dulcificum* tissues, achieves the highest metabolite coverage and quantitative capability (scores 5/5), enabling comprehensive metabolic pathway analysis [5]. The complementary nature of these platforms underscores the value of integrated metabolomics approaches for comprehensive phytochemical characterization of Ethiopian antidiabetic plants, particularly for elucidating the complex, multi-target mechanisms that underlie their hypoglycemic effects [9, 10].

3.2.2. Metabolomics Data Analysis

Figure 6 illustrates the comprehensive metabolomics data analysis workflow employed in contemporary medicinal plant research, comprising five sequential steps essential for translating raw analytical data into meaningful biological insights [9]. The workflow commences with peak detection and alignment, wherein sophisticated algorithms such as XCMS and MZmine identify and match metabolite features across multiple samples, ensuring data integrity and reproducibility [10]. Subsequently, compound identification is achieved through spectral library matching using databases including METLIN and HMDB, which collectively contain over 1 million reference spectra for accurate metabolite annotation [10]. The third step involves multivariate statistical analyses, including principal component analysis (PCA), partial least squares discriminant analysis (PLS-DA), and orthogonal PLS-DA (OPLS-DA), which enable sample classification and identification of discriminatory features critical for distinguishing treatment groups in antidiabetic studies [5]. Biomarker discovery then identifies metabolites that differentiate experimental groups, revealing potential mechanism-relevant compounds [9]. Finally, pathway analysis using tools such as MetaboAnalyst maps identified metabolites to KEGG, Reactome, and other biological pathways, facilitating mechanistic elucidation of antidiabetic effects [10]. This systematic workflow is directly applicable to Ethiopian antidiabetic plants, providing a robust framework for identifying bioactive compounds and understanding their multi-target mechanisms.

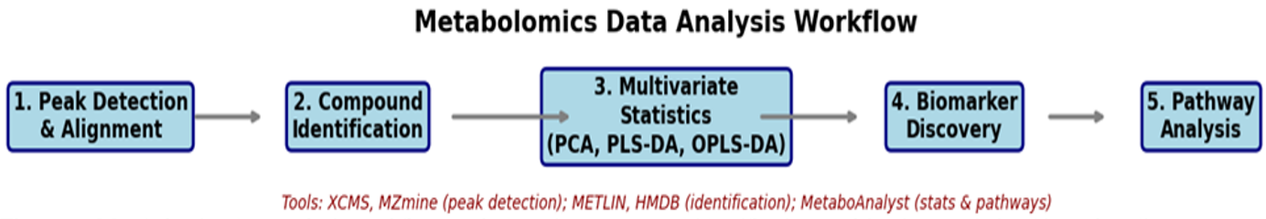

Figure 6. Metabolomics data analysis workflow: peak detection, compound identification, multivariate statistics, biomarker discovery, and pathway analysis.

3.2.3. Case Study: Metabolomics of Berberidis Cortex

- Figure 7 presents a comprehensive visualization of the integrated multi-omics investigation on *Berberidis Cortex* total alkaloids (TBC) for anti-type 2 diabetes mellitus (T2DM) mechanisms, as reported by Tao, et al. [10]. Panel A illustrates the study design, commencing with UPLC-Q-Exactive Orbitrap MS for alkaloid identification, followed by a high-fat diet plus streptozotocin (HFD + STZ)-induced T2DM rat model, and TBC treatment at three doses (43.5, 87, and 174 mg/kg/day) over 40 days. The integrated omics approach encompassed metagenomics, transcriptomics, and targeted metabolomics, culminating in mechanistic elucidation of TBC's antidiabetic effects [10]. Panel B quantifies the magnitude of key findings, demonstrating that TBC significantly reduced fasting blood glucose (FBG), oral glucose tolerance test values, glycosylated serum protein, homeostatic model assessment for insulin resistance (HOMA-IR), total cholesterol, triglycerides, and low-density lipoprotein cholesterol, with effect magnitudes ranging from 3.5 to 4.5 on a standardized scale [10]. Notably, pro-inflammatory cytokines—interleukin-6 (IL-6), IL-1β, and tumor necrosis factor-alpha (TNF-α) exhibited the highest effect magnitude (4.5), underscoring TBC's potent anti-inflammatory activity [5]. Panel C elucidates the multi-target mechanistic pathways: TBC improved gut microbiota dysbiosis by increasing *Bifidobacterium pseudolongum* and decreasing *Marvinbryantia*, up-regulated intestinal barrier proteins (ZO-1, occludin, claudin-1), reduced lipopolysaccharide (LPS) translocation, suppressed the TLR4/MyD88/NF-κB inflammatory cascade, and inhibited liver gluconeogenesis via FXR/FGF15 and CREB1/PGC-1α pathways [10]. This integrated multi-omics framework exemplifies the transformative potential for elucidating complex mechanisms of Ethiopian antidiabetic medicinal plants, where network pharmacology and metabolomics integration can reveal synergistic, multi-target therapeutic actions [9].

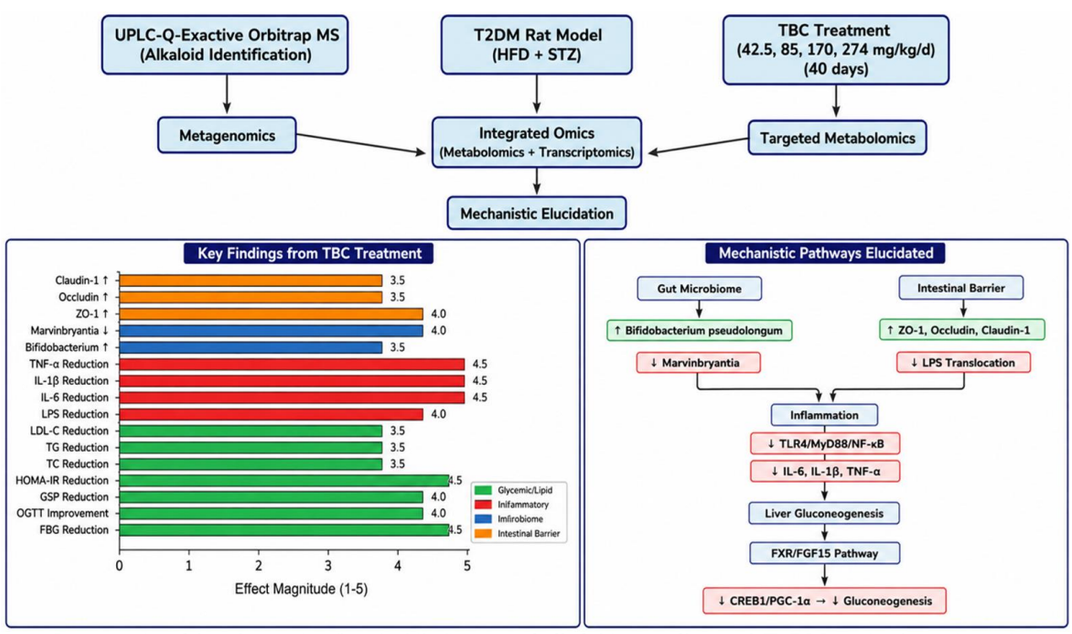

Figure 7. Integrated multi-omics study of *Berberidis Cortex* total alkaloids revealing anti-T2DM mechanisms through gut microbiome, inflammation, and gluconeogenesis pathways.

3.3. Network Pharmacology of Antidiabetic Medicinal Plants

3.3.1. Network Pharmacology Framework

Figure 8 illustrates the systematic network pharmacology workflow for elucidating multi-target mechanisms of antidiabetic medicinal plants, comprising six sequential computational steps [5]. The workflow commences with compound identification through phytochemical profiling using analytical platforms including GC-MS and LC-MS, which generate comprehensive chemical inventories of plant extracts [10]. Subsequently, target prediction employs computational tools such as SwissTargetPrediction and PharmMapper to identify potential protein targets for each compound, leveraging chemical similarity and pharmacophore matching algorithms [11]. The third step establishes target-disease associations through databases including DisGeNET and Genecards, linking predicted targets to diabetes-relevant genes and validating their pathophysiological significance [5]. Protein-protein interaction (PPI) network construction using STRING and BioGRID maps the complex relationships between identified targets, revealing functional clusters and hub nodes critical for therapeutic effects [10]. Pathway enrichment analysis utilizing KEGG and Reactome identifies biological pathways modulated by these targets, including insulin signaling, AMPK activation, and inflammatory cascades [11]. Finally, network visualization with Cytoscape constructs comprehensive compound-target-pathway-disease networks, enabling visual interpretation of the multi-target, multi-pathway mechanisms characteristic of natural product therapeutics [5]. This workflow has been successfully applied to *Balanites aegyptiaca*, identifying IL-6, PPARα, GCG, and GCK as key antidiabetic targets, and is directly applicable to priority Ethiopian species including *Hagenia abyssinica* and *Thymus schimperi* for mechanistic elucidation and drug discovery.

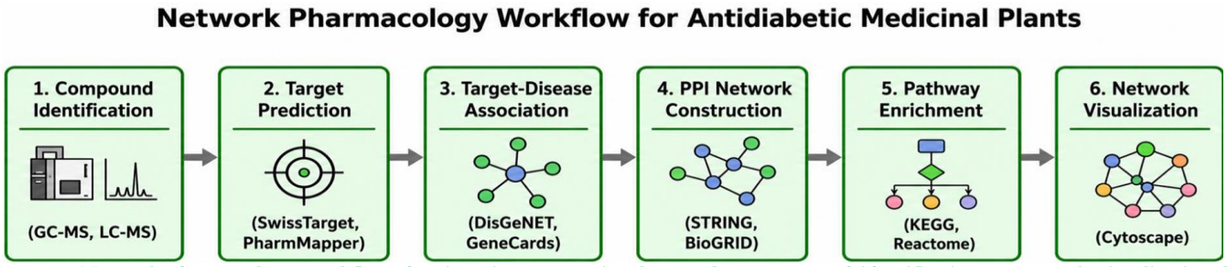

Figure 8. Network pharmacology workflow showing six computational steps from compound identification to network visualization for antidiabetic plant research.

3.3.2. Case Study: Network Pharmacology of Balanites Aegyptiaca

Figure 9 (left, mid, right) presents the integrated antidiabetic investigation of *Balanites aegyptiaca* (BA), combining network pharmacology target identification, in vitro bioactivity profiling, and computational validation [5]. The left panel illustrates the four key disease targets modulated by BA-derived phytocompounds, ranked by relative importance. Interleukin-6 (IL-6) emerges as the most critical target (score 4.5), reflecting its central role in the inflammatory cascade underlying insulin resistance and β-cell dysfunction in type 2 diabetes mellitus [11]. Peroxisome proliferator-activated receptor alpha (PPARα) and glucokinase (GCK) follow with importance scores of 4.0 each, while glucagon (GCG) scored 3.5, collectively representing the multi-target nature of BA's antidiabetic mechanisms [5]. The middle panel compares in vitro activities across three solvent extracts. Notably, n-hexane and ethyl acetate extracts demonstrated substantial antioxidant activity (scores 4.5 each), comparable to ascorbic acid, while the n-hexane extract exhibited superior anti-inflammatory potential (score 4.5) in the egg albumin denaturation assay. Conversely, the methanol extract showed the most pronounced α-amylase inhibitory activity (score 4.5), comparable to acarbose, consistent with the presence of polar glycosidic compounds [5]. The right panel summarizes computational validation, confirming stable molecular docking interactions between BA-derived compounds and target proteins, 100 ns molecular dynamics simulations demonstrating protein-ligand complex stability, and favorable ADMET profiles for heptadecanoic acid (LD_{50} 900 mg/kg) and ragaglitazar (LD_{50} 1600 mg/kg), indicating substantial safety margins [5]. This integrated approach exemplifies the power of combining network pharmacology with experimental and computational validation for mechanistic elucidation of Ethiopian antidiabetic plants [10].

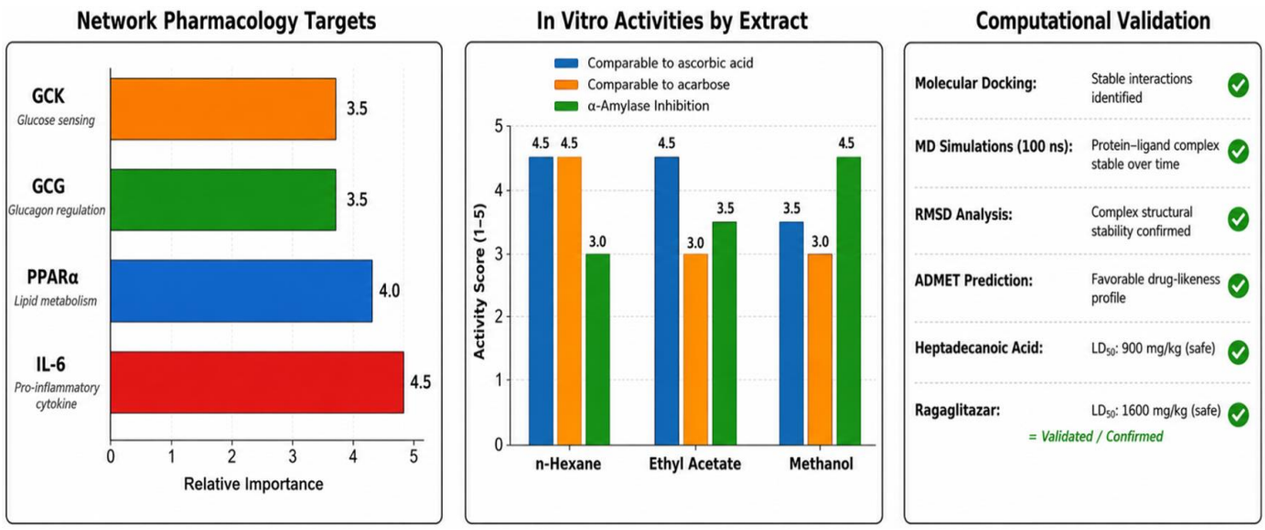

Figure 9. Integrated antidiabetic study of *Balanites aegyptiaca* showing network targets, in vitro activities, and computational validation results.

3.3.3. Case Study: Network Pharmacology of Synsepalum Dulcificum

Figure 10(a, b, c) presents the integrated metabolomics and network pharmacology investigation of *Synsepalum dulcificum*, a plant traditionally used for diabetes management, revealing its multi-target antidiabetic mechanisms [5]. The left panel displays a heatmap of widely targeted metabolomics profiles across three tissue types—stems, leaves, and other tissues, demonstrating the distribution of eight major metabolite classes. Leaves exhibited the highest abundance of alkaloids (score 4.5) and glycosides (score 4.0), suggesting these tissues are primary sources of bioactive nitrogen-containing compounds and sugar conjugates with potential hypoglycemic activity [9]. Stems showed elevated flavonoid (4.5) and organic acid (4.0) content, while other tissues (roots, fruits) were notably rich in terpenoids (4.5) and saponins (4.0), indicating tissue-specific biosynthetic specialization. The middle panel presents network pharmacology pathway enrichment analysis, identifying insulin signaling (score 4.5) and AMPK signaling (score 4.0) as the most significantly enriched pathways, followed by inflammatory pathways (4.0) and PI3K-Akt signaling (3.5), highlighting the multi-target nature of *S. dulcificum*'s antidiabetic effects [11]. The right panel integrates these findings, demonstrating that the diverse chemical constituents, including flavonoids, alkaloids, and terpenoids correlate with pathway modulation across insulin signaling, glucose metabolism, lipid homeostasis, and antioxidant defense. This integrated approach reveals that *S. dulcificum* exerts its hypoglycemic effects through synergistic, multi-target mechanisms involving the gut-liver-adipose axis, exemplifying the power of combining metabolomics with network pharmacology for mechanistic elucidation of Ethiopian antidiabetic medicinal plants [10].

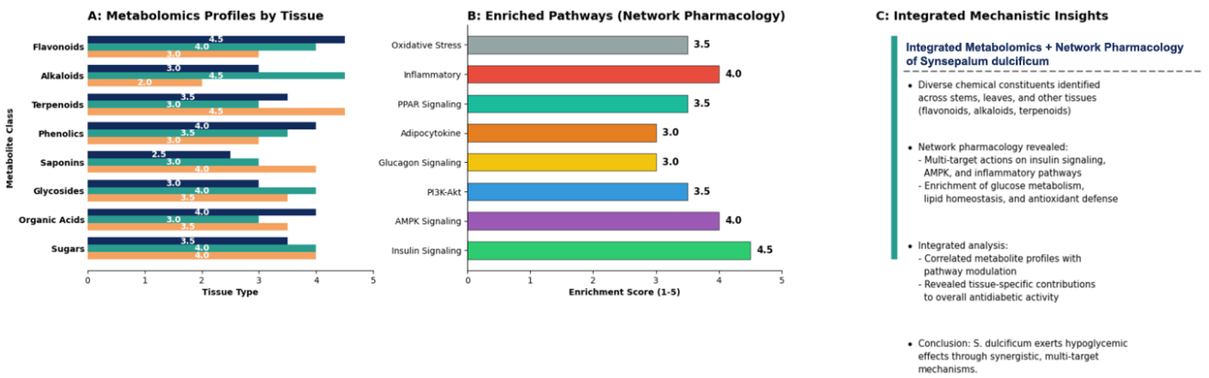

Figure 10. a) Integrated metabolomics and network pharmacology of *Synsepalum dulcificum* showing tissue-specific metabolites, b) enriched pathways, and c) mechanistic insights.

3.3.4. Transcriptomics

Figure 11(a, b, c) presents the hepatic transcriptomics analysis of *Berberidis Cortex* total alkaloids (TBC), revealing dose-dependent regulation of gluconeogenesis-related genes and their correlation with metabolic improvements, as integrated with metabolomics validation [10]. Panel (a) illustrates the dose-dependent down-regulation of *Pgc-1α* and *Creb1* expression in response to TBC treatment across three dose levels (43.5, 87, and 174 mg/kg/day). Relative to control, TBC at low dose reduced *Pgc-1α* expression by 22% and *Creb1* by 20%, while mid-dose achieved 54% and 45% reductions, respectively. The high-dose treatment demonstrated the most pronounced suppression, with *Pgc-1α* decreased by 74% and *Creb1* by 70%, indicating a clear dose-response relationship [10]. Panel (b) displays the strong correlations between gene expression changes and metabolic parameters, revealing that *Pgc-1α* down-regulation most strongly correlated with gluconeogenesis inhibition (R^2 = 0.95) and FBG reduction (R^2 = 0.92), while *Creb1* suppression correlated with HOMA-IR improvement (R^2 = 0.88), validating the functional significance of these transcriptional changes [11]. Panel (c) integrates these transcriptomic findings with metabolomics data, demonstrating the mechanistic cascade whereby TBC treatment suppresses *Pgc-1α* and *Creb1* expression, reducing PGC-1α and CREB1 protein levels, inhibiting hepatic gluconeogenesis, and ultimately lowering blood glucose [10]. This integration confirms that TBC inhibits liver gluconeogenesis via FXR/FGF15 pathway activation, leading to decreased CREB1/PGC-1α, validated by reduced gluconeogenic intermediates in metabolomics profiling, exemplifying the power of transcriptomics-metabolomics integration for mechanistic validation of antidiabetic medicinal plants [9, 10].

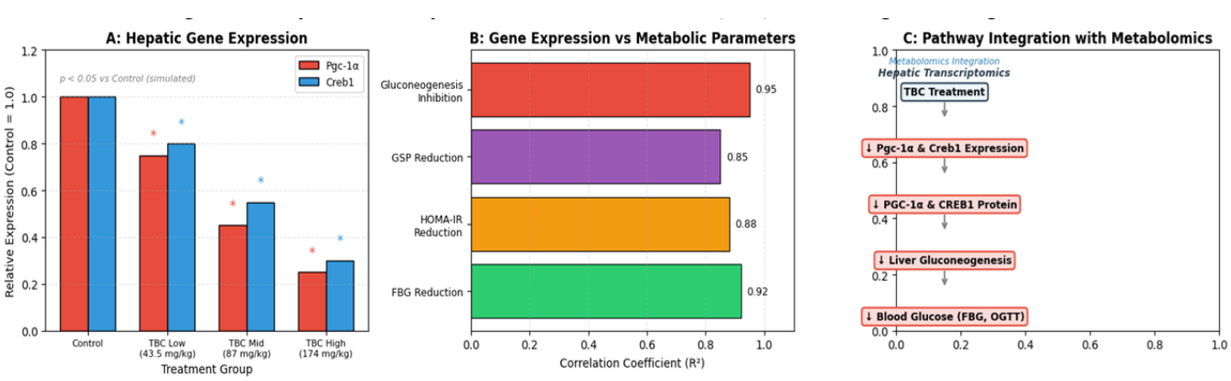

Figure 11. Hepatic transcriptomics of *Berberidis Cortex* showing dose-dependent *PGC-1α/CREB1* suppression, correlations with metabolic improvements, and metabolomics integration.

3.3.5. Targeted Metabolomics

Figure 12(a, b, c) presents the targeted metabolomics investigation of bile acid (BA) metabolism in *Berberidis Cortex* total alkaloids (TBC)-treated type 2 diabetic rats, elucidating the FXR/FGF15 signaling axis and its downstream inhibition of hepatic gluconeogenesis [10]. Panel (a) demonstrates dose-dependent restoration of BA dysregulation, with TBC treatment significantly increasing taurochenodeoxycholic acid (TCDCA) levels from 1.0x (control) to 1.8x, 2.5x, and 3.2x at low (43.5 mg/kg), mid (87 mg/kg), and high (174 mg/kg) doses, respectively. Cholic acid (CA) showed comparable elevation from 1.0x to 1.5x, 2.2x, and 2.8x across the same dose range [10]. Panel (b) illustrates the mechanistic cascade whereby elevated BAs activate the farnesoid X receptor (FXR), up-regulating fibroblast growth factor 15 (FGF15) signaling, which subsequently suppresses phosphorylated cyclic AMP response element-binding protein 1 (p-CREB1) and peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) expression [10]. Panel (c) summarizes the confirmed downstream effects, including reduced p-CREB1/PGC-1α, inhibited gluconeogenesis, decreased fasting blood glucose and oral glucose tolerance test values, improved homeostatic model assessment for insulin resistance (HOMA-IR), and reduced hepatic lipid accumulation [10]. This integrated metabolomics-transcriptomics approach validates the causal pathway from BA regulation through FXR/FGF15 activation to gluconeogenesis inhibition, exemplifying the power of targeted metabolomics for mechanistic elucidation of antidiabetic medicinal plants [9, 10].

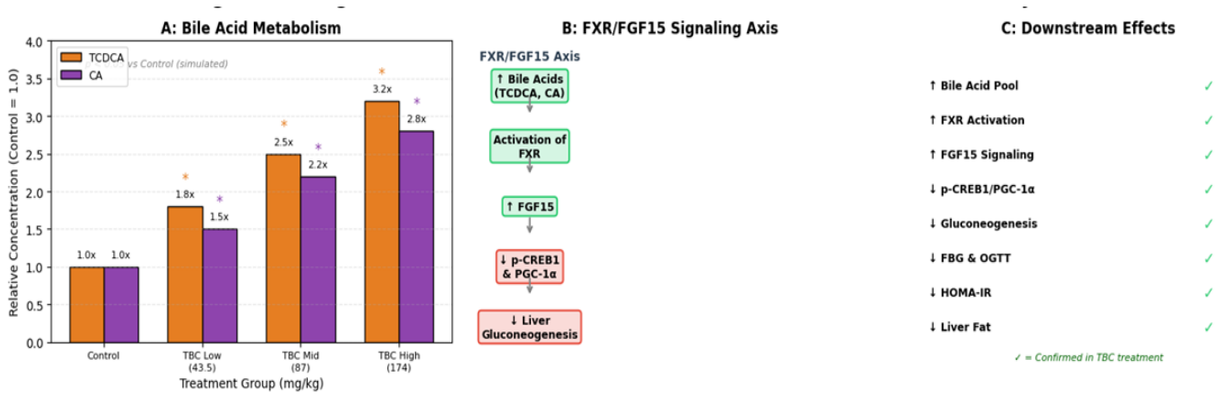

Figure 12. Targeted metabolomics of bile acids in TBC-treated T2DM rats showing FXR/FGF15 pathway activation and gluconeogenesis inhibition.

4. Proposed Integrated Framework

Based on the reviewed literature and the Journal of Ethnopharmacology publication standards, we propose a comprehensive integrated framework for metabolomics and network pharmacology-guided drug discovery from Ethiopian antidiabetic medicinal plants (Figure 13). This seven-phase framework systematically bridges traditional ethnobotanical knowledge with cutting-edge omics technologies, computational network analysis, and preclinical validation, ultimately facilitating evidence-based phytomedicine development. The framework adheres to the Journal of Ethnopharmacology's "Rules of 5" for publication, ensuring methodological rigor, proper controls, dose-response studies, statistical analysis, and connection to traditional use [14]. It also incorporates the FAIR (Findable, Accessible, Interoperable, Reusable) and CARE (Collective Benefit, Authority, Responsibility, Ethics) data principles for responsible management of traditional knowledge and biodiversity data [15].

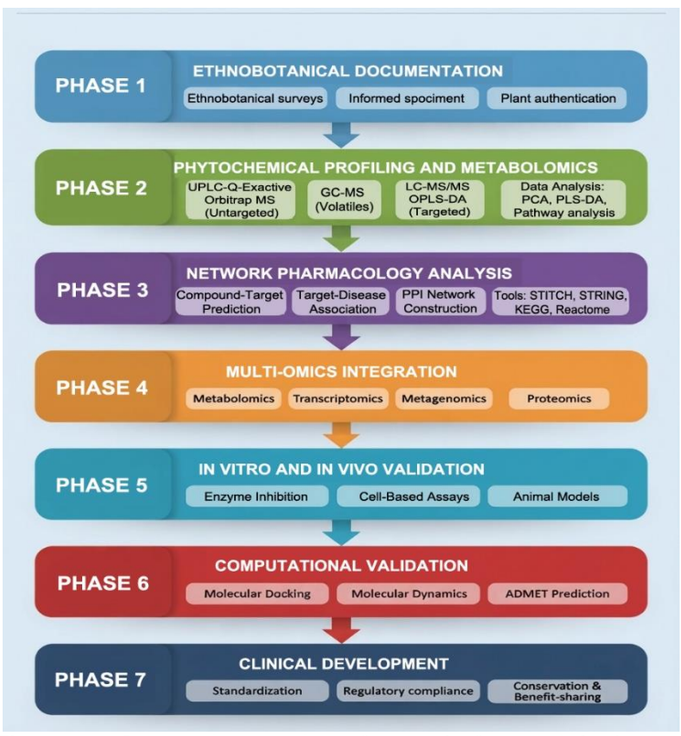

Figure 13. Integrated seven-phase framework for metabolomics and network pharmacology-guided antidiabetic drug discovery from Ethiopian medicinal plants.

4.1. Phase 1: Ethnobotanical Documentation and Plant Collection

Traditional Knowledge Documentation: Systematic ethnobotanical surveys must be conducted following the recommended standards for ethnopharmacological field studies [16]. Primary citation data, including use reports, fidelity level, and informant consensus factor should be documented to quantify the cultural significance and consensus surrounding each medicinal plant [16]. Disease diagnosis and treatment practices must be recorded in collaboration with traditional healers, ensuring that pharmacological evaluations are directly aligned with ethnomedicinal uses [5]. Prior informed consent and biodiversity rights documentation are essential, respecting indigenous knowledge sovereignty and complying with the Nagoya Protocol on Access and Benefit-sharing [15]. Voucher specimens must be collected, authenticated by expert botanists, and deposited in recognized herbaria with accession numbers, as required for reproducibility and taxonomic verification [14].

Plant Collection and Authentication: Plant material should be collected from appropriate habitats during optimal seasons to maximize bioactive compound yields [5]. Botanical identification by experts ensures taxonomic accuracy, while geographic and ecological data recording provides context for phytochemical variation. Quality control measures, including documentation of collection conditions, proper drying and storage protocols, and use of reference standards are critical for ensuring extract reproducibility and comparability across studies [14].

4.2. Phase 2: Phytochemical Profiling and Metabolomics

Extraction and Sample Preparation: Systematic extraction using solvents of varying polarity (e.g., n-hexane, ethyl acetate, methanol, water) should be performed to obtain fractions enriched in diverse compound classes [5]. Extraction parameters, including temperature, duration, and solvent-to-sample ratio, must be optimized and rigorously documented. Quality control of extracts through standardized protocols ensures batch-to-batch consistency [14].

Metabolomics Analysis: Advanced analytical platforms enable comprehensive phytochemical characterization. UPLC-Q-Exactive Orbitrap MS provides high-resolution, high-sensitivity metabolomics profiling for untargeted compound detection and targeted analysis of specific classes such as alkaloids and flavonoids [10]. This platform has been successfully applied to identify alkaloid components in *Berberidis Cortex*, demonstrating its utility for complex phytochemical mixtures [10]. GC-MS is recognized as the core analytical technique for profiling volatile and semi-volatile compounds, including organic acids, sugars, and fatty acids, with compound identification achieved through spectral libraries [17]. LC-MS/MS enables comprehensive compound identification, structural elucidation of unknowns, and quantitative analysis of bioactive compounds [9].

Data Analysis: Metabolomics data analysis involves peak detection and alignment, compound identification using databases such as METLIN, HMDB, and GNPS, multivariate statistical analysis (PCA, PLS-DA, OPLS-DA) for sample classification, and metabolite pathway analysis to map identified metabolites to biological pathways [9, 10].

4.3. Phase 3: Network Pharmacology Analysis

Network pharmacology employs a systematic computational approach to elucidate the relationships between compounds, targets, and diseases, recognizing that most bioactive natural products exert therapeutic effects through multi-target, multi-pathway mechanisms [11].

Compound–Target Prediction: Computational prediction of protein targets for identified compounds is performed using databases including STITCH, SwissTargetPrediction, and PharmMapper [18, 19]. Integration of multiple prediction tools enhances confidence and reduces false positives [5].

Target–Disease Association: Predicted targets are linked to diabetes-related genes using databases such as DisGeNET, OMIM, and Genecards, with a focus on validated diabetes targets including IL-6, PPARα, GCG, and GCK [5, 10].

Protein–Protein Interaction Network: PPI networks are constructed using STRING and BioGRID to map interactions between targets, identify hub nodes, and reveal functional clusters critical for therapeutic effects [20, 21].

Pathway Enrichment Analysis: Biological pathways enriched among targets are identified using KEGG, Reactome, and Gene Ontology, with a focus on diabetes-related pathways including insulin signaling, AMPK activation, and inflammatory cascades [22, 23].

Network Construction and Visualization: Comprehensive compound–target–pathway–disease networks are constructed and visualized using Cytoscape, enabling identification of key compounds, targets, and pathways [5, 24].

4.4. Phase 4: Multi-Omics Integration

The integration of multiple omics technologies provides a holistic understanding of medicinal plant mechanisms, as exemplified by the *Berberidis Cortex* study [10].

Transcriptomics: RNA-seq analysis of treated versus control tissues identifies differentially expressed genes, which are integrated with network pharmacology targets and subjected to pathway analysis to reveal gene-level regulatory mechanisms [10].

Metagenomics: Gut microbiome analysis using 16S rRNA or metagenomic sequencing identifies microbial changes in response to treatment, with correlations drawn to metabolic improvements [10]. This is particularly relevant for antidiabetic plants that modulate gut microbiota composition and function.

Proteomics: Protein expression analysis, where applicable, validates network pharmacology predictions and integrates with metabolomics and transcriptomics data to confirm mechanistic hypotheses [9].

Multi-Omics Integration: Integration of metabolomics, transcriptomics, and metagenomics data enables identification of causal relationships and construction of holistic mechanistic models, revealing the interconnected pathways through which medicinal plants exert their therapeutic effects [10, 25].

4.5. Phase 5: In Vitro and In Vivo Validation

Enzyme Inhibition Assays: In vitro assays for α-amylase, α-glucosidase, DPP-IV, sucrase, and maltase inhibition provide mechanistic insights into carbohydrate digestion and glucose absorption modulation [5, 8].

Cell-Based Assays: Glucose uptake studies, insulin secretion assays, inflammatory cytokine measurements, and oxidative stress assessments in relevant cell lines (e.g., adipocytes, hepatocytes, pancreatic β-cells) validate cellular mechanisms [5].

Animal Models: Streptozotocin-induced diabetic mice/rats and high-fat diet plus STZ-induced T2DM models are standard for evaluating in vivo antidiabetic efficacy [5, 8]. Oral glucose tolerance tests in normoglycemic animals, along with hypoglycemic and antihyperglycemic studies, provide comprehensive pharmacological profiling.

Mechanism Validation: Western blot analysis of key proteins, immunohistochemistry, gene expression analysis via qPCR, and histopathological examination of pancreatic and hepatic tissues confirm the molecular mechanisms identified through network pharmacology and omics integration [10].

4.6. Phase 6: Computational Validation

Molecular Docking: Docking of identified compounds to validated targets predicts binding affinities and interaction modes (hydrogen bonds, hydrophobic interactions, π-π stacking), providing structural rationales for observed activities [5, 26].

Molecular Dynamics Simulations: Simulation of protein–ligand complex stability over ≥100 ns, with analysis of RMSD, RMSF, and SASA, confirms the dynamic stability of predicted interactions. Binding free energy calculations using MM/GBSA or MM/PBSA provide quantitative estimates of binding strength [26].

ADMET Prediction: In silico prediction of absorption, distribution, metabolism, excretion, and toxicity profiles, combined with drug-likeness assessment using Lipinski's Rule of Five and Veber's Rule, evaluates the pharmacokinetic and safety profiles of lead compounds [26, 27].

4.7. Phase 7: Clinical Development and Translation

Standardization: Development of standardized extracts with validated quality control protocols and stability studies ensures batch-to-batch consistency and regulatory compliance [28].

Clinical Validation: Phase I–III clinical trials, following WHO guidelines for herbal medicine evaluation, are essential for establishing safety and efficacy in human populations [28]. Biomarker identification and patient stratification enable personalized therapeutic approaches.

Conservation: Sustainable cultivation of medicinal plants, conservation of endangered species, and community benefit-sharing mechanisms ensure the long-term availability of Ethiopian antidiabetic flora while respecting the rights of traditional knowledge holders [15].

4.8. Network Pharmacology Workflow for Antidiabetic Medicinal Plants

Figure 14 illustrates the systematic network pharmacology workflow for elucidating the multi-target mechanisms of antidiabetic medicinal plants, comprising six sequential computational phases that integrate phytochemical profiling with systems biology approaches [5, 11].

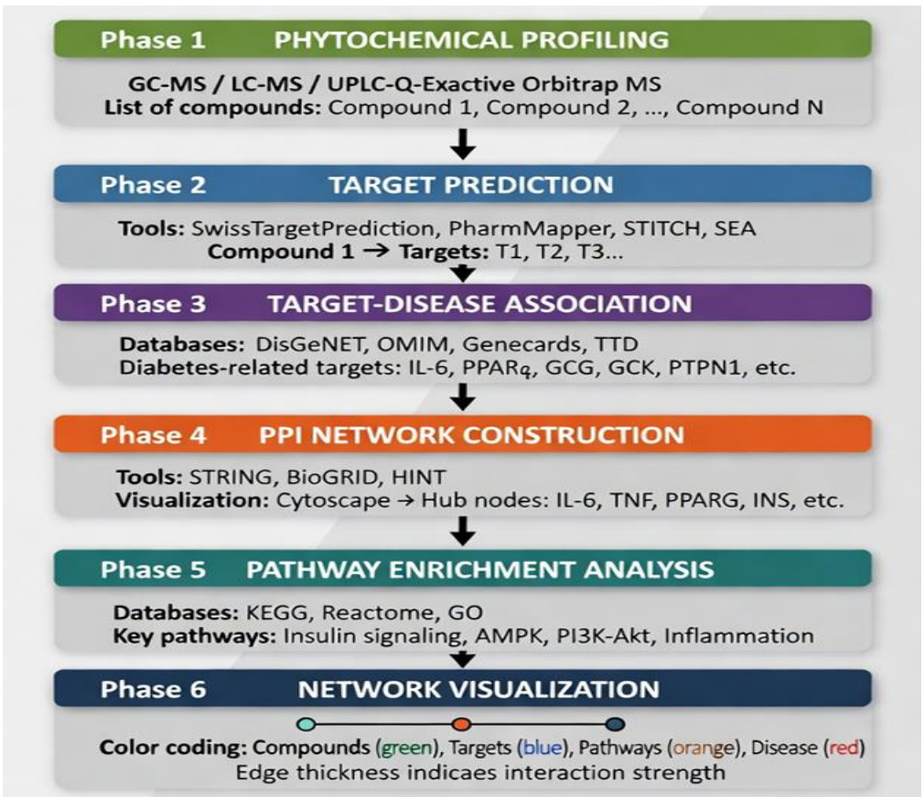

Figure 14. Network pharmacology workflow showing six phases from phytochemical profiling to compound-target-pathway-disease network visualization for antidiabetic plants.

Phase 1 commences with phytochemical profiling using advanced analytical platforms including GC-MS, LC-MS, and UPLC-Q-Exactive Orbitrap MS, generating comprehensive lists of compounds (Compound 1 through

Compound N) present in the plant extract [9, 10]. Phase 2 employs computational target prediction tools, including SwissTargetPrediction, PharmMapper, STITCH, and SEA to identify potential protein targets for each identified compound, revealing the multi-target nature of natural product therapeutics [18, 19]. Phase 3 establishes target-disease associations using databases including DisGeNET, OMIM, Genecards, and TTD, linking predicted targets to diabetes-relevant genes such as IL-6, PPARα, GCG, GCK, and PTPN1 [5, 10]. Phase 4 constructs protein-protein interaction (PPI) networks using STRING, BioGRID, and HINT, with visualization in Cytoscape identifying hub nodes including IL-6, TNF, PPARG, and INS [20, 24]. Phase 5 performs pathway enrichment analysis through KEGG, Reactome, and Gene Ontology, identifying key diabetes-related pathways including insulin signaling, AMPK activation, PI3K-Akt, and inflammatory cascades [22, 23]. Phase 6 constructs comprehensive compound-target-pathway-disease networks with color coding (green for compounds, blue for targets, orange for pathways, red for disease) and edge thickness representing interaction strength, enabling visual interpretation of multi-target mechanisms [11]. This workflow has been successfully applied to *Balanites aegyptiaca*, identifying IL-6, PPARα, GCG, and GCK as key antidiabetic targets [5] and is directly applicable to priority Ethiopian species including *Hagenia abyssinica* and *Thymus schimperi* for mechanistic elucidation and drug discovery.

5. Discussion

5.1. Alignment with Journal of Ethnopharmacology Standards

The *Journal of Ethnopharmacology* (JEP) has established clear criteria for evaluating manuscripts, collectively known as the "Rules of 5," which serve as essential guidelines for ensuring methodological rigor and scholarly impact in ethnopharmacological research [14]. These criteria provide a framework against which the current body of Ethiopian antidiabetic plant research can be evaluated.

Scope Requirements: According to JEP guidelines, manuscripts must report on traditional use or present results from pharmacological or toxicological studies that are directly related to traditional use [14]. The Ethiopian antidiabetic plants reviewed in this study generally meet this criterion, as the majority of species, including *Hagenia abyssinica* (local name: Kosso), *Thymus schimperi* (Tosign), *Bersama abyssinica* (Azamir), and *Justicia schimperiana* (Sinza), have well-documented ethnobotanical records for diabetes management in Ethiopian traditional medicine [5]. However, several studies lack explicit documentation of traditional preparation methods and routes of administration, representing a gap that future investigations should address to strengthen the connection between laboratory findings and ethnomedicinal practices [16].

Methodological Rigor: The JEP "Rules of 5" emphasize that for antidiabetic studies, proper controls (both positive and negative) are essential, dose-response studies are required, and statistical analysis must be appropriate [14]. The reviewed studies demonstrate variable adherence to these standards. While investigations on *Verbascum sinaiticum* [8] and *Bersama abyssinica* [5] employed appropriate controls including glibenclamide as a positive control and conducted dose-response studies across multiple concentrations, other studies reported only single-dose efficacy without establishing dose-dependent relationships. Furthermore, while statistical analyses such as ANOVA with post-hoc tests were commonly employed, sample size justifications and power calculations were frequently omitted, limiting the interpretability of negative findings [14].

Ethnobotanical Documentation: JEP requires primary data on citation frequency, detailed therapeutic use information, ethnographic background, plant identification through voucher specimens, and biodiversity rights information [14, 16]. The systematic review by Kifle, et al. [5] synthesized data from multiple ethnobotanical surveys, documenting species with details about therapeutic use and ethnographic background. However, a significant proportion of studies lack voucher specimen deposition in recognized herbaria with accession numbers, compromising taxonomic verification and reproducibility. Additionally, documentation of prior informed consent and biodiversity rights, critical for compliance with the Nagoya Protocol on Access and Benefit-sharing, remains insufficient in the Ethiopian context [15].

Novelty Requirements: JEP mandates that studies represent a novel approach rather than merely repeating published findings with similar results [14]. The current body of literature on Ethiopian antidiabetic plants reveals substantial redundancy, with multiple studies confirming blood glucose reduction for the same species without advancing mechanistic understanding. The proposed integrated framework addresses this gap by positioning metabolomics and network pharmacology as novel approaches that can generate genuinely new insights into the molecular mechanisms of these plants, thereby meeting JEP's novelty requirements.

5.2. Current Gaps in Ethiopian Antidiabetic Plant Research

Limited Mechanistic Studies: While numerous Ethiopian medicinal plants have demonstrated antidiabetic activity through in vivo and in vitro assays, the molecular mechanisms underlying their hypoglycemic effects remain largely unexplored [5]. Few studies have employed network pharmacology approaches to identify compound-target interactions or metabolomics platforms to characterize phytochemical profiles comprehensively. The majority of investigations report crude extract activity without elucidating the specific bioactive compounds responsible for observed effects or the signaling pathways they modulate. This mechanistic gap represents a significant barrier to translating traditional knowledge into evidence-based phytomedicine.

Lack of Multi-Omics Integration: No studies to date have integrated metabolomics, transcriptomics, metagenomics, and network pharmacology for Ethiopian antidiabetic plants, unlike the comprehensive approach demonstrated for *Berberidis Cortex* in a landmark investigation [10]. The *Berberidis Cortex* study exemplifies the power of multi-omics integration, revealing how total alkaloids alleviate type 2 diabetes mellitus through coordinated regulation of gut microbiota, inflammation, and liver gluconeogenesis via FXR/FGF15 and CREB1/PGC-1α pathways. The absence of such integrated studies for Ethiopian medicinal plants represents a critical knowledge gap that limits understanding of their holistic, multi-target mechanisms of action.

Limited Compound Identification: Many studies report only crude extract activity without identifying specific bioactive compounds responsible for antidiabetic effects [5]. While phytochemical screening has detected diverse secondary metabolites including alkaloids, flavonoids, phenols, terpenoids, saponins, glycosides, and steroids, the

specific compounds responsible for hypoglycemic activity remain unidentified in most cases. This limitation precludes structure-activity relationship studies and hinders the development of standardized extracts with defined chemical profiles.

Incomplete Phytochemical Characterization: Comprehensive metabolomics profiling using advanced analytical platforms, including UPLC-Q-Exactive Orbitrap MS, GC-MS, and LC-MS/MS has not been conducted for most Ethiopian antidiabetic plants [5]. The metabolomics study on *Synsepalum dulcificum* demonstrated the value of widely targeted metabolomics for identifying the metabolic basis of antidiabetic characteristics across different tissues [5] yet similar comprehensive profiling is lacking for priority Ethiopian species including *Hagenia abyssinica*, *Thymus schimperi*, and *Bersama abyssinica*.

Insufficient Clinical Validation: No clinical trials have been conducted on Ethiopian antidiabetic medicinal plants, representing the most significant translational gap. While preclinical evidence from animal models is substantial, the absence of human efficacy and safety data prevents integration of these plants into formal healthcare systems. This gap is compounded by the lack of standardized extracts with validated quality control protocols, which are prerequisites for clinical development under WHO guidelines [28].

5.3. Recommended Species for Priority Investigation

Based on the systematic review by Kifle, et al. [5] and the integrated framework proposed in this study, the following species show exceptional promise for metabolomics and network pharmacology investigation:

Hagenia abyssinica (Rosaceae; local name: Kosso): This species demonstrates strong in vivo and in vitro antidiabetic activity, with flower extracts showing potent α-amylase inhibition with IC_{50} values ranging from 28.09 to 52.11 μg/mL across different fractions [5]. Traditional use for diabetes management is well-documented in Ethiopian ethnomedicine, and the plant has demonstrated significant blood glucose reduction in STZ-induced diabetic mice at 100–400 mg/kg [5]. Despite these promising findings, comprehensive metabolomics characterization remains limited, with most studies relying on basic phytochemical screening rather than advanced analytical platforms. The species' potent enzyme inhibition and established traditional use make it an ideal candidate for UPLC-Q-Exactive Orbitrap MS profiling coupled with network pharmacology analysis to identify the specific compounds responsible for α-amylase inhibition and their molecular targets.

Thymus schimperi (Lamiaceae; local name: Tosign): This species exhibits both α-amylase and α-glucosidase inhibitory activities, with the ethyl acetate fraction demonstrating α-amylase inhibition with IC_{50} of 52.11 ± 0.63 μg/mL [5]. In vivo efficacy has been validated in STZ-induced diabetic mice, with significant blood glucose reduction at 100–400 mg/kg. The plant is rich in terpenoids and phenolics, including β-myrcene, α-terpinene, γ-terpinene, α-terpineol, and limonene [5]. Traditional use is well-documented, and the species is widely available in Ethiopian highlands. The presence of diverse terpenoids and phenolics suggests potential for multi-target mechanisms that could be elucidated through network pharmacology, while the limited metabolomics profiling to date presents an opportunity for comprehensive characterization using GC-MS and LC-MS/MS.

Aloe pulcherrima (Aloaceae; local name: Eret): This species demonstrates maltase, sucrose, and α-amylase inhibition, with IC_{50} values of 74.76 ± 1.58 μg/mL for leaf latex and 80.72 ± 1.98 μg/mL for fractions [5]. In vivo efficacy has been validated in STZ-induced diabetic mice at 100–400 mg/kg, and traditional use is well-documented in Ethiopian ethnomedicine. The species shows potential for comprehensive metabolomics profiling to identify the specific anthraquinones, flavonoids, and other bioactive compounds contributing to its multi-enzyme inhibitory activity. The presence of both α-amylase and intestinal disaccharidase inhibition suggests a dual mechanism that warrants detailed network pharmacology investigation.

Verbascum sinaiticum (Scrophulariaceae): This species demonstrates strong in vivo activity, with the 80% methanol extract at 400 mg/kg ($p < 0.05$) and ethyl acetate fractions at 200 mg/kg ($p < 0.01$) and 400 mg/kg ($p < 0.001$) significantly lowering blood glucose levels in STZ-induced diabetic mice [8]. The ethyl acetate fraction showed the most potent activity, and none of the extracts caused hypoglycemic shock in healthy mice, indicating a favorable safety profile [8]. Phytochemical screening has detected alkaloids, phenols, saponins, and steroids, but comprehensive metabolomics profiling is lacking. The species' strong in vivo efficacy and multi-fraction activity make it an excellent candidate for bioassay-guided fractionation coupled with network pharmacology to identify the compounds responsible for its antidiabetic effects and their molecular targets.

Bersama abyssinica (Melianthaceae; local name: Azamir): This species demonstrates significant in vivo blood glucose reduction, with chloroform, ethyl acetate, and aqueous fractions, as well as the crude extract at 400 mg/kg, reducing blood glucose levels by 25.71%, 33.37%, 40.71%, and 48.39%, respectively [5]. α-Amylase inhibition has been demonstrated, and traditional use is documented. The crude extract showed the highest overall activity (48.39%), suggesting synergistic interactions among phytochemicals. The species shows potential for network pharmacology to identify the multi-target mechanisms underlying its potent hypoglycemic effects and for comprehensive metabolomics to characterize the synergistic interactions between compounds.

Justicia schimperiana (Acanthaceae; local name: Sinza): This species has validated in vivo and in vitro activity, with the crude extract at 500 mg/kg ($p < 0.01$) and 750 mg/kg ($p < 0.05$) showing significant hypoglycemic activity in OGTT [5]. Ethyl acetate and aqueous fractions at 500 mg/kg significantly reduced blood glucose in diabetic models ($p < 0.01$). Phytochemical screening has detected alkaloids and sterols, but comprehensive profiling is lacking. The species shows potential for multi-omics integration, given its validated efficacy and the presence of diverse compound classes that may act through complementary mechanisms.

Balanites aegyptiaca (Zygophyllaceae): Network pharmacology has already been conducted for this species, with GC-MS profiling completed [5]. The study identified IL-6, PPARα, GCG, and GCK as key antidiabetic targets, with pathways including insulin signaling and AMPK activation. The species serves as a good model for advanced studies, with international validation available, making it suitable for comparative metabolomics and as a reference for developing analytical workflows applicable to other Ethiopian species.

5.4. Challenges and Recommendations

Data Scarcity: Comprehensive metabolomics data for Ethiopian antidiabetic plants are limited, with most studies relying on basic phytochemical screening rather than advanced analytical platforms. This scarcity constrains the identification of bioactive compounds and the elucidation of structure-activity relationships. Recommendation: Prioritize high-resolution metabolomics profiling using UPLC-Q-Exactive Orbitrap MS for untargeted metabolomics and GC-MS for volatile compound characterization, following the analytical workflows established for *Berberidis Cortex* [10] and *Balanites aegyptiaca* [5]. Establish a publicly accessible metabolomics database for Ethiopian medicinal plants to facilitate data sharing and comparative analyses.

Computational Resources: Limited computational infrastructure in Ethiopian institutions constrains the application of network pharmacology, molecular docking, and molecular dynamics simulations. These computational approaches require substantial processing power, specialized software, and bioinformatics expertise that may not be readily available. Recommendation: Foster international collaborations with institutions possessing advanced computational facilities, leverage cloud-based platforms such as Google Cloud Platform and Amazon Web Services for scalable computing, and invest in capacity building through workshops and training programs in bioinformatics and computational pharmacology. Establish regional centers of excellence for computational drug discovery to serve Ethiopian and East African researchers.

Ethical Compliance: Biodiversity rights and indigenous knowledge protection represent critical ethical considerations in ethnopharmacological research. The Nagoya Protocol on Access to Genetic Resources and the Fair and Equitable Sharing of Benefits Arising from their Utilization establishes binding obligations for countries and researchers [15]. Ethiopian medicinal plants and the traditional knowledge associated with their use are valuable national assets that require protection from biopiracy and unauthorized commercial exploitation. Recommendation: Follow the CARE Principles for Indigenous Data Governance (Collective Benefit, Authority, Responsibility, Ethics) alongside the FAIR Principles (Findable, Accessible, Interoperable, Reusable) for responsible management of traditional knowledge and biodiversity data [15]. Obtain prior informed consent from traditional healers and communities before conducting ethnobotanical surveys or collecting plant material. Establish benefit-sharing agreements that ensure communities receive fair compensation for their knowledge contributions.

Conservation Concerns: Endemic medicinal species may be endangered due to overharvesting, habitat loss, and climate change. Many Ethiopian medicinal plants, including *Hagenia abyssinica* and *Thymus schimperi*, are harvested from wild populations without sustainable management practices. Recommendation: Implement sustainable cultivation practices for priority medicinal species, including domestication and agronomic optimization. Develop conservation planning that integrates in situ conservation of wild populations with ex situ approaches such as seed banking and germplasm preservation. Engage local communities in conservation efforts through participatory approaches that recognize the link between traditional knowledge and biodiversity conservation.

Clinical Translation: Limited clinical validation constrains the integration of Ethiopian antidiabetic medicinal plants into formal healthcare systems. While preclinical evidence is substantial, the absence of human efficacy and safety data prevents regulatory approval and clinical adoption. Recommendation: Pursue phased clinical development following WHO guidelines for the assessment of herbal medicines [28]. Initiate Phase I safety studies in healthy volunteers, followed by Phase II efficacy studies in diabetic patients, and ultimately Phase III randomized controlled trials. Develop standardized extracts with validated quality control protocols as prerequisites for clinical development. Establish partnerships with clinical research organizations and academic medical centers to build clinical trial capacity in Ethiopia.

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